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National Institutes of Health

Guidelines for Research Involving Recombinant DNA Molecules

August 1982

These NIH Guidelines supersede earlier versions and will be in effect until further notice.

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I. SCOPE OF THE GUIDELINES

I-A. Purpose. The purpose of these Guidelines is to specify practices for constructing and handling (i) recombinant DNA molecules and (ii) organisms and viruses containing recombinant DNA molecules.

I-B. Definition of Recombinant DNA Molecules. In the context of these Guidelines, recombinant DNA molecules are defined as either (i) molecules which are constructed outside living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell, or (ii) DNA molecules that result from the replication of those described in (i) above.

Synthetic DNA segments likely to yield a potentially harmful polynucleotide or polypeptide (e.g., a toxin or a pharmacologically active agent) shall be considered as equivalent to their natural DNA counterpart. If the synthetic DNA segment is not expressed *in vivo* as a biologically active polynucleotide or polypeptide product, it is exempt from the Guidelines.

I-C. General Applicability. The Guidelines are applicable to all recombinant DNA research within the United States or its territories which is conducted at or sponsored by an Institution that receives any support for recombinant DNA research from NIH. This includes research performed by NIH directly.

An individual receiving support for research involving recombinant DNA must be associated with or sponsored by an Institution that can and does assume the responsibilities assigned in these Guidelines.

The Guidelines are also applicable to projects done abroad if they are supported by NIH funds. If the host country, however, has established rules for the conduct of recombinant DNA projects, then a certificate of compliance with those rules may be submitted to NIH in lieu of compliance with the NIH Guidelines. NIH reserves the right to withhold funding if the safety practices to be employed abroad are not reasonably consistent with the NIH Guidelines.

I-D. General Definitions. The following terms, which are used throughout the Guidelines, are defined as follows:

IV-D-1. "Institution" means any public or private entity (including Federal, State, and local government agencies).

I-D-2. "Institutional Biosafety Committee" or "IBC" means a committee that (i) meets the requirements for membership specified in Section IV-B-2, and (ii) reviews, approves, and oversees projects in accordance with the responsibilities defined in Sections IV-B-2 and IV-B-3.

I-D-3. "NIH Office of Recombinant DNA Activities" or "ORDA" means the office within NIH with responsibility for (i) reviewing and coordinating all activities of NIH related to the Guidelines, and (ii) performing other duties as defined in Section IV-C-3.

I-D-4. "Recombinant DNA Advisory Committee" or "RAC" means the public advisory committee that advises the Secretary, the Assistant Secretary for Health, and the Director of the National Institutes of Health concerning recombinant DNA research. The RAC shall be constituted as specified in Section IV-C-2.

I-D-5. "Director, NIH" or "Director" means the Director of the National Institutes of Health or any other officer or employee of NIH to whom authority has been delegated.

II. CONTAINMENT

Effective biological safety programs have been operative in a variety of laboratories for many years. Considerable information, therefore, already exists for the design of physical containment facilities and the selection of laboratory procedures applicable to organisms carrying recombinant DNAs [3-16]. The existing programs rely upon mechanisms that, for convenience, can be divided into two categories: (i) a set of standard practices that are generally used in microbiological laboratories, and (ii) special procedures, equipment, and laboratory installations that provide physical barriers which are applied in varying degrees according to the estimated biohazard. Four levels of physical containment, which are designated as P1, P2, P3, and P4 are described in Appendix G. P4 provides the most stringent containment conditions, P1 the least stringent.

Experiments on recombinant DNAs, by their very nature, lend themselves to a third containment mechanism—namely, the application of highly specific biological barriers. In fact, natural barriers do exist which limit either (i) the infectivity of a vector, or vehicle, (plasmid or virus) for specific hosts or (ii) its dissemination and survival in the environment. The vectors that provide the means for replication of the recombinant DNAs and/or the host cells in which they replicate can be genetically designed to decrease

by many orders of magnitude the probability of dissemination of recombinant DNAs outside the laboratory. Further details on biological containment may be found in Appendix I.

As these three means of containment are complementary, different levels of containment appropriate for experiments with different recombinants can be established by applying various combinations of the physical and biological barriers along with a constant use of the standard practices. We consider these categories of containment separately in order that such combinations can be conveniently expressed in the Guidelines.

In constructing these Guidelines, it was necessary to define boundary conditions for the different levels of physical and biological containment and for the classes of experiments to which they apply. We recognize that these definitions do not take into account all existing and anticipated information on special procedures that will allow particular experiments to be carried out under different conditions than indicated here without affecting risk. Indeed, we urge that individual investigators devise simple and more effective containment procedures and that investigators and institutional biosafety committees recommend changes in the Guidelines to permit their use.

III. CONTAINMENT GUIDELINES FOR COVERED EXPERIMENTS

Part III discusses experiments involving recombinant DNA. These experiments have been divided into four classes:

III-A. Experiments which require specific RAC review and NIH and IBC approval before initiation of the experiment;

III-B. Experiments which require IBC approval before initiation of the experiment;

III-C. Experiments which require IBC notification at the time of initiation of the experiment;

III-D. Experiments which are exempt from the procedures of the Guidelines.

IF AN EXPERIMENT FALLS INTO BOTH CLASS III-A AND ONE OF THE OTHER CLASSES, THE RULES PERTAINING TO CLASS III-A MUST BE FOLLOWED. If an experiment falls into class III-D and into either class III-B or III-C as well, it can be considered exempt from the requirements of the Guidelines.

Changes in containment levels from those specified here may not be instituted without the express approval of the Director, NIH. (See Sections IV-C-1-b-(1), IV-C-1-b-(2), and subsections.)

III-A. Experiments that Require RAC Review and NIH and IBC Approval Before Initiation. Experiments in this category cannot be initiated without submission of relevant information on the proposed experiment to NIH, the publication of the proposal in the Federal Register for thirty days of comment, review by the RAC, and specific approval by NIH. The containment conditions for such experiments will be recommended by RAC and set by NIH at the time of approval. Such experiments also require the approval of the IBC before initiation. Specific experiments already approved in this section and the appropriate containment conditions are listed in Appendices D and F. If an experiment is similar to those listed in Appendices D and F, ORDA may determine appropriate containment conditions according to case precedents under Section IV-C-1-b-(3)-(g).

III-A-1. Deliberate formation of recombinant DNAs containing genes for the biosynthesis of toxic molecules lethal for vertebrates at an LD_{50} of less than 100 nanograms per kilogram body weight (e.g., microbial toxins such as the botulinum toxins, tetanus toxin, diphtheria toxin, Shigella dysenteriae neurotoxin). Specific approval has been given for the cloning in E. coli K-12 of DNAs containing genes coding for the biosynthesis of toxic molecules which are lethal to vertebrates at 100 nanograms to 100 micrograms per kilogram body weight. Containment levels for these experiments are specified in Appendix F.

III-A-2. Deliberate release into the environment of any organism containing recombinant DNA.

III-A-3. Deliberate transfer of a drug resistance trait to microorganisms that are not known to acquire it naturally [2], if such acquisition could compromise the use of the drug to control disease agents in human or veterinary medicine or agriculture.

III-B. Experiments that Require IBC Approval Before Initiation. Investigators performing experiments in this category must submit to their Institutional Biosafety Committee (IBC), prior to initiation of the experiments, a registration document that contains a description of: (a) the source(s) of DNA, (b) the nature of the inserted DNA sequences, (c) the hosts and vectors to be used, (d) whether a deliberate attempt will be made to obtain expression of a foreign gene, and, if so, what protein will be produced, and (e) the containment conditions specified in these Guidelines. This registration document must be dated and signed by the investigator and filed only with the local IBC. The IBC shall review all such proposals prior to initiation of the experiments. Requests for lowering of containment for experiments in this category will be considered by NIH. (See Section IV-C-1-b-(3).)

III-B-1. Experiments Using Human or Animal Pathogens (Class 2, Class 3, Class 4, or Class 5 Agents [1]) as Host-Vector Systems.

III-B-1-a. Experiments involving the introduction of recombinant DNA into Class 2 agents can be carried out at P2 containment.

III-B-1-b. Experiments involving the introduction of recombinant DNA into Class 3 agents can be carried out at P3 containment.

III-B-1-c. Experiments involving the introduction of recombinant DNA into Class 4 agents can be carried out at P4 containment.

III-B-1-d. Containment conditions for experiments involving the introduction of recombinant DNA into Class 5 agents will be set on a case-by-case basis following ORDA review. A USDA permit is required for work with Class 5 agents [18, 20].

III-B-2. Experiments in Which DNA from Human or Animal Pathogens (Class 2, Class 3, Class 4, or Class 5 Agents [1]) is Cloned in Nonpathogenic Prokaryotic or Lower Eukaryotic Host-Vector Systems.

III-B-2-a. Recombinant DNA experiments in which DNA from Class 2 or Class 3 agents [1] is transferred into nonpathogenic prokaryotes or lower eukaryotes may be performed under P2 containment. Recombinant DNA experiments in which DNA from Class 4 agents is transferred into nonpathogenic prokaryotes or lower eukaryotes can be performed at P2 containment after

demonstration that only a totally and irreversibly defective fraction of the agent's genome is present in a given recombinant. In the absence of such a demonstration, P4 containment should be used. Specific lowering of containment to P1 for particular experiments can be approved by the IBC. Many experiments in this category will be exempt from the Guidelines. (See Sections III-D-4 and III-D-5.) Experiments involving the formation of recombinant DNAs for certain genes coding for molecules toxic for vertebrates require RAC review and NIH approval (see Section III-A-1), or must be carried out under NIH specified conditions as described in Appendix F.

III-B-2-b. Containment conditions for experiments in which DNA from Class 5 agents is transferred into nonpathogenic prokaryotes or lower eukaryotes will be determined by ORDA following a case-by-case review. A USDA permit is required for work with Class 5 agents [18, 20].

III-B-3. Experiments Involving the Use of Infectious Animal or Plant Viruses or Defective Animal or Plant Viruses in the Presence of Helper Virus in Tissue Culture Systems.

CAUTION: Special care should be used in the evaluation of containment levels for experiments which are likely to either enhance the pathogenicity (e.g., insertion of a host oncogene) or to extend the host range (e.g., introduction of novel control elements) of viral vectors under conditions which permit a productive infection. In such cases, serious consideration should be given to raising the physical containment by at least one level.

NOTE: Recombinant DNA molecules which contain less than two-thirds of the genome of any eukaryotic virus (all virus from a single Family [17] being considered identical [19]) may be considered defective and can be used, in the absence of helper, under the conditions specified in Section III-C.

III-B-3-a. Experiments involving the use of infectious Class 2 animal viruses [1], or defective Class 2 animal viruses in the presence of helper virus, can be performed at P2 containment.

III-B-3-b. Experiments involving the use of infectious Class 3 animal viruses [1], or defective Class 3 animal viruses in the presence of helper virus, can be carried out at P3 containment.

III-B-3-c. Experiments involving the use of infectious Class 4 viruses [1], or defective Class 4 viruses in the presence of helper virus, may be carried out under P4 containment.

III-B-3-d. Experiments involving the use of infectious Class 5 [1] viruses, or defective Class 5 viruses in the presence of helper virus will be determined on a case-by-case basis following ORDA review. A USDA permit is required for work with Class 5 pathogens [18, 20].

III-B-3-e. Experiments involving the use of infectious animal or plant viruses, or defective animal or plant viruses in the presence of helper virus, not covered by Sections III-B-3-a, III-B-3-b, III-B-3-c, or III-B-3-d may be carried out under P1 containment.

III-B-4. Recombinant DNA Experiments Involving Whole Animals or Plants.

III-B-4-a. DNA from any source except for greater than two-thirds of a eukaryotic viral genome may be transferred to any non-human vertebrate organism and propagated under conditions of physical containment comparable to P1 and appropriate to the organism under study [2]. It is important that the investigator demonstrate that the fraction of the viral genome being utilized does not lead to productive infection. A USDA permit is required for work with Class 5 agents [18,20].

III-B-4-b. For all experiments involving whole animals and plants and not covered by III-B-4-a, the appropriate containment will be determined by the IBC.

III-B-5. Experiments Involving More than 10 Liters of Culture. The appropriate containment will be decided by the IBC. Where appropriate, the large-scale containment recommendations of the NIH should be used (45 FR 24968).

III-C. Experiments that Require IBC Notice Simultaneously with Initiation of Experiments. Experiments not included in Sections III-A, III-B, III-D, and subsections of these Sections are to be considered in Section III-C. All such experiments can be carried out at P1 containment. For experiments in this category, a registration document as described in Section III-B must be dated and signed by the investigator and filed with the local IBC. The IBC shall

review all such proposals, but IBC review prior to initiation of the experiment is not required. (The reader should refer to the policy statement in the first two paragraphs of Section IV-A.)

For example, experiments in which all components derive from non-pathogenic prokaryotes and non-pathogenic lower eukaryotes fall under Section III-C and can be carried out at P1 containment.

CAUTION: Experiments Involving Formation of Recombinant DNA Molecules Containing no more Than Two-Thirds of the Genome of any Eukaryotic Virus. Recombinant DNA molecules containing no more than two-thirds of the genome of any eukaryotic virus (all viruses from a single Family [17] being considered identical [19]) may be propagated and maintained in cells in tissue culture using P1 containment. For such experiments, it must be shown that the cells lack helper virus for the specific Families of defective viruses being used. If helper virus is present, procedures specified under Section III-B-3 should be used. The DNA may contain fragments of the genome of viruses from more than one Family but each fragment must be less than two-thirds of a genome.

III-D. Exempt Experiments. The following recombinant DNA molecules are exempt from these Guidelines and no registration with the IBC is necessary.

III-D-1. Those that are not in organisms or viruses.

III-D-2. Those that consist entirely of DNA segments from a single non-chromosomal or viral DNA source, though one or more of the segments may be a synthetic equivalent.

III-D-3. Those that consist entirely of DNA from a prokaryotic host, including its indigenous plasmids or viruses, when propagated only in that host (or a closely related strain of the same species) or when transferred to another host by well established physiological means; also, those that consist entirely of DNA from an eukaryotic host, including its chloroplasts, mitochondria, or plasmids (but excluding viruses), when propagated only in that host (or a closely related strain of same species).

III-D-4. Certain specified recombinant DNA molecules that consist entirely of DNA segments from different species that exchange DNA by known physiological processes, though one or more of the segments may be a synthetic equivalent. A list of such exchangers will be prepared and periodically revised by the Director, NIH, with advice of the RAC, after appropriate notice and opportunity for public comment. (See Section IV-C-1-b-(1)-(c).) Certain classes are exempt as of publication of these Revised Guidelines. The list is in Appendix A. An updated list may be obtained from the Office of Recombinant DNA Activities, National Institutes of Health, Bethesda, Maryland 20205.

III-D-5. Other classes of recombinant DNA molecules, if the Director, NIH, with advice of the RAC, after appropriate notice and opportunity for public comment, finds that they do not present a significant risk to health or the environment. (See Section IV-C-1-b-(1)-(c).) Certain classes are

exempt as of publication of these Revised Guidelines. The list is in Appendix C. An updated list may be obtained from the Office of Recombinant DNA Activities, National Institutes of Health, Bethesda, Maryland 20205.

IV. ROLES AND RESPONSIBILITIES

IV-A. Policy. Safety in activities involving recombinant DNA depends on the individual conducting them. The Guidelines cannot anticipate every possible situation. Motivation and good judgement are the key essentials to protection of health and the environment.

The Guidelines are intended to help the Institution, the Institutional Biosafety Committee (IBC), the Biological Safety Officer, and the Principal Investigator determine the safeguards that should be implemented. These Guidelines will never be complete or final, since all conceivable experiments involving recombinant DNA cannot be foreseen. Therefore, it is the responsibility of the Institution and those associated with it to adhere to the intent of the Guidelines as well as to their specifics.

Each Institution (and the IBC acting on its behalf) is responsible for ensuring that recombinant DNA activities comply with the Guidelines. General recognition of institutional authority and responsibility properly establishes accountability for safe conduct of the research at the local level.

The following roles and responsibilities constitute an administrative framework in which safety is an essential and integral part of research involving recombinant DNA molecules. Further clarifications and interpretations of roles and responsibilities will be issued by NIH as necessary.

IV-B. Responsibilities of the Institution.

IV-B-1. General Information. Each Institution conducting or sponsoring recombinant DNA research covered by these Guidelines is responsible for ensuring that the research is carried out in full conformity with the provisions of the Guidelines. In order to fulfill this responsibility, the Institution shall:

IV-B-1-a. Establish and implement policies that provide for the safe conduct of recombinant DNA research and that ensure compliance with the Guidelines. The Institution, as part of its general responsibilities for implementing the Guidelines, may establish additional procedures, as deemed necessary, to govern the Institution and its components in the discharge of its responsibilities under the Guidelines. This may include (i) statements formulated by the Institution for general implementation of the Guidelines and (ii) whatever additional precautionary steps the Institution may deem appropriate.

IV-B-1-b. Establish an Institutional Biosafety Committee (IBC) that meets the requirements set forth in Section IV-B-2 and carries out the functions detailed in Section IV-B-3.

IV-B-1-c. If the Institution is engaged in recombinant DNA research at the P3 or P4 containment level, appoint a Biological Safety Officer (BSO), who shall be a member of the IBC and carry out the duties specified in Section IV-B-4.

IV-B-1-d. Require that investigators responsible for research covered by these Guidelines comply with the provisions of Section IV-B-5, and assist investigators to do so.

IV-B-1-e. Ensure appropriate training for the IBC chairperson and members, the BSO, Principal Investigators (PIs), and laboratory staff regarding the Guidelines, their implementation, and laboratory safety. Responsibility for training IBC members may be carried out through the IBC chairperson. Responsibility for training laboratory staff may be carried out through the PI. The Institution is responsible for seeing that the PI has sufficient training, but may delegate this responsibility to the IBC.

IV-B-1-f. Determine the necessity, in connection with each project, for health surveillance of recombinant DNA research personnel, and conduct, if found appropriate, a health surveillance program for the project. [The Laboratory Safety Monograph (LSM) discusses various possible components of such a program--for example, records of agents handled, active investigation of relevant illnesses, and the maintenance of serial serum samples for monitoring serologic changes that may result from the employees' work experience. Certain medical conditions may place a laboratory worker at increased risk in any endeavor where infectious agents are handled. Examples given in the LSM include gastrointestinal disorders and treatment with steroids, immunosuppressive drugs, or antibiotics. Workers with such

disorders or treatment should be evaluated to determine whether they should be engaged in research with potentially hazardous organisms during their treatment or illness. Copies of the LSM are available from ORDA.]

IV-B-1-g. Report within 30 days to ORDA any significant problems with and violations of the Guidelines and significant research-related accidents and illnesses, unless the institution determines that the PI or IBC has done so.

IV-B-2. Membership and Procedures of the IBC. The Institution shall establish an Institutional Biosafety Committee (IBC) whose responsibilities need not be restricted to recombinant DNA. The committee shall meet the following requirements:

IV-B-2-a. The IBC shall comprise no fewer than five members so selected thay they collectively have experience and expertise in recombinant DNA technology and the capability to assess the safety of recombinant DNA research experiments and any potential risk to public health or the environment. At least two members shall not be affiliated with the Institution (apart from their membership on the IBC) and shall represent the interest of the surrounding community with respect to health and protection of the environment. Members meet this requirement if, for example, they are officials of State or local public health or environmental protection agencies, members of other local governmental bodies, or persons active in medical, occupational health, or

environmental concerns in the community. The Biological Safety Officer (BSO), mandatory when research is being conducted at the P3 and P4 levels, shall be a member. (See Section IV-B-4.)

IV-B-2-b. In order to ensure the competence necessary to review recombinant DNA activities, it is recommended that (i) the IBC include persons with expertise in recombinant DNA technology, biological safety, and physical containment; (ii) the IBC include, or have available as consultants, persons knowledgeable in institutional commitments and policies, applicable law, standards of professional conduct and practice, community attitudes, and the environment; and (iii) at least one member be from the laboratory technical staff.

IV-B-2-c. The Institution shall identify the committee members by name in a report to the NIH Office of Recombinant DNA Activities (ORDA) and shall include relevant background information on each member in such form and at such times as ORDA may require.

IV-B-2-d. No member of an IBC may be involved (except to provide information requested by the IBC) in the review or approval of a project in which he or she has been, or expects to be, engaged or has a direct financial interest.

IV-B-2-e. The Institution, who is ultimately responsible for the effectiveness of the IBC, may establish procedures that the IBC will follow in its initial and continuing review of applications, proposals, and activities. (IBC review procedures are specified in Section IV-B-3-a.)

IV-B-2-f. Institutions are encouraged to open IBC meetings to the public whenever possible, consistent with protection of privacy and proprietary interests.

IV-B-2-g. Upon request, the Institution shall make available to the public all minutes of IBC meetings and any documents submitted to or received from funding agencies which the latter are required to make available to the public. If comments are made by members of the public on IBC actions, the Institution shall forward to NIH both the comments and the IBC's response.

IV-B-3. Functions of the IBC. On behalf of the Institution, the IBC is responsible for:

IV-B-3-a. Reviewing for compliance with the NIH Guidelines recombinant DNA research as specified in Part III conducted at or sponsored by the Institution, and approving those research projects that it finds are in conformity with the Guidelines. This review shall include:

IV-B-3-a-(1). An independent assessment of the containment levels required by these Guidelines for the proposed research, and

IV-B-3-a-(2). An assessment of the facilities, procedures, and practices, and of the training and expertise of recombinant DNA personnel.

IV-B-3-b. Notifying the Principal Investigator (PI) of the results of their review.

IV-B-3-c. Lowering containment levels for certain experiments as specified in Sections III-B-2.

IV-B-3-d. Setting containment levels as specified in Section III-B-4-b and III-B-5.

IV-B-3-e. Reviewing periodically recombinant DNA research being conducted at the Institution, to ensure that the requirements of the Guidelines are being fulfilled.

IV-B-3-f. Adopting emergency plans covering accidental spills and personnel contamination resulting from such research.

NOTE: Basic elements in developing specific procedures for dealing with major spills of potentially hazardous materials in the laboratory are detailed in the Laboratory Safety Monograph (LSM). Included are information and references on decontamination and emergency plans. NIH and the Centers for Disease Control are available to provide consultation, and direct assistance

if necessary, as posted in the LSM. The Institution shall cooperate with the State and local public health departments, reporting any significant research-related illness or accident that appears to be a hazard to the public health.

IV-B-3-g. Reporting within 30 days to the appropriate institutional official and to the NIH Office of Recombinant DNA Activities (ORDA) any significant problems with or violations of the Guidelines, and any significant research-related accidents or illnesses, unless the IBC determines that the PI has done so.

IV-B-3-h. The IBC may not authorize initiation of experiments not explicitly covered by the Guidelines until NIH (with the advice of the RAC when required) establishes the containment requirement.

IV-B-3-i. Performing such other functions as may be delegated to the IBC under Section IV-B-1.

IV-B-4. Biological Safety Officer. The Institution shall appoint a BSO if it engages in recombinant DNA research at the P3 or P4 containment level. The officer shall be a member of the Institutional Biosafety Committee (IBC), and his or her duties shall include (but need not be limited to):

IV-B-4-a. Ensuring through periodic inspections that laboratory standards are rigorously followed;

IV-B-4-b. Reporting to the IBC and the Institution all significant problems with and violations of the Guidelines and all significant research-related accidents and illnesses of which the BSO becomes aware, unless the BSO determines that the Principal Investigator (PI) has done so;

IV-B-4-c. Developing emergency plans for dealing with accidental spills and personnel contamination, and investigating recombinant DNA research laboratory accidents;

IV-B-4-d. Providing advice on laboratory security;

IV-B-4-e. Providing technical advice to the PI and the IBC on research safety procedures.

NOTE: See Laboratory Safety Monograph for additional information on the duties of the BSO.

IV-B-5. Principal Investigator. On behalf of the Institution, the PI is responsible for complying fully with the Guidelines in conducting any recombinant DNA research.

IV-B-5-a. PI—General. As part of this general responsibility, the PI shall:

IV-B-5-a-(1). Initiate or modify no recombinant DNA research requiring approval by the IBC prior to initiation (see Sections III-A and III-B) until that research, or the proposed modification thereof, has been approved by the IBC and has met all other requirements of the Guidelines;

IV-B-5-a-(2). Determine whether experiments are covered by Section III-C and follow the appropriate procedures;

IV-B-5-a-(3). Report within 30 days to the IBC and NIH (ORDA) all significant problems with and violations of the Guidelines and all significant research-related accidents and illnesses;

IV-B-5-a-(4). Report to the IBC and to NIH (ORDA) new information bearing on the Guidelines;

IV-B-5-a-(5). Be adequately trained in good microbiological techniques;

IV-B-5-a-(6). Adhere to IBC-approved emergency plans for dealing with accidental spills and personnel contamination; and

IV-B-5-a-(7). Comply with shipping requirements for recombinant DNA molecules. (See Appendix H for shipping requirements and the Laboratory Safety Monograph for technical recommendations.)

IV-B-5-b. Submissions by the PI to NIH. The PI shall:

IV-B-5-b-(1). Submit information to NIH (ORDA) in order to have new host-vector systems certified;

IV-B-5-b-(2). Petition NIH, with notice to the IBC, for exemptions to these Guidelines;

IV-B-5-b-(3). Petition NIH, with concurrence of the IBC, for approval to conduct experiments specified in Section III-A of the Guidelines;

IV-B-5-b-(4). Petition NIH for determination of containment for experiments requiring case-by-case review;

IV-B-5-b-(5). Petition NIH for determination of containment for experiments not covered by the Guidelines.

IV-B-5-c. Submissions by the PI to the IBC. The PI shall:

IV-B-5-c-(1). Make the initial determination of the required levels of physical and biological containment in accordance with the Guidelines;

IV-B-5-c-(2). Select appropriate microbiological practices and laboratory techniques to be used in the research;

IV-B-5-c-(3). Submit the initial research protocol if covered under Guidelines Sections III-A, III-B, or III-C (and also subsequent changes--e.g., changes in the source of DNA or host-vector system) to the IBC for review and approval or disapproval; and

IV-B-5-c-(4). Remain in communication with the IBC throughout the conduct of the project.

IV-B-5-d. PI Responsibilities Prior to Initiating Research. The PI is responsible for:

IV-B-5-d-(1). Making available to the laboratory staff copies of the protocols that describe the potential biohazards and the precautions to be taken;

IV-B-5-d-(2). Instructing and training staff in the practices and techniques required to ensure safety and in the procedures for dealing with accidents; and

IV-B-5-d-(3). Informing the staff of the reasons and provisions for any precautionary medical practices advised or requested, such as vaccinations or serum collection.

IV-B-5-e. PI Responsibilities During the Conduct of the Research. The PI is responsible for:

IV-B-5-e-(1). Supervising the safety performance of the staff to ensure that the required safety practices and techniques are employed;

IV-B-5-e-(2). Investigating and reporting in writing to ORDA, the Biological Safety Officer (where applicable), and the IBC any significant problems pertaining to the operation and implementation of containment practices and procedures;

IV-B-5-e-(3). Correcting work errors and conditions that may result in the release of recombinant DNA materials;

IV-B-5-e-(4). Ensuring the integrity of the physical containment (e.g., biological safety cabinets) and the biological containment (e.g., purity, and genotypic and phenotypic characteristics).

IV-C. Responsibilities of NIH.

IV-C-1. Director. The Director, NIH, is responsible for (i) establishing the NIH Guidelines for Research Involving Recombinant DNA Molecules, (ii) overseeing their implementation, and (iii) their final interpretation.

The Director has responsibilities under the Guidelines that involve the NIH Office of Recombinant DNA Activities (ORDA) and the Recombinant DNA Advisory Committee (RAC). ORDA's responsibilities under the Guidelines are administrative. Advice from the RAC is primarily scientific and technical. In certain circumstances, there is specific opportunity for public comment, with published response, before final action.

IV-C-1-a. General Responsibilities of the Director, NIH. The responsibilities of the Director shall include the following:

IV-C-1-a-(1). Promulgating requirements as necessary to implement the Guidelines;

IV-C-1-a-(2). Establishing and maintaining the RAC to carry out the responsibilities set forth in Section IV-C-2. The RAC's membership is specified in its charter and in Section IV-C-2;

IV-C-1-a-(3). Establishing and maintaining ORDA to carry out the responsibilities defined in Section IV-C-3; and

IV-C-1-a-(4). Maintaining the Federal Interagency Advisory Committee on Recombinant DNA Research established by the Secretary, HEW (now HHS), for advice on the coordination of all Federal programs and activities relating to recombinant DNA, including activities of the RAC. (See Appendix J.)

IV-C-1-b. Specific Responsibilities of the Director, NIH. In carrying out the responsibilities set forth in this Section, the Director or a designee shall weigh each proposed action, through appropriate analysis and consultation, to determine that it complies with the Guidelines and presents no significant risk to health or the environment.

IV-C-1-b-(1). Major Actions. To execute major actions the Director must seek the advice of the RAC and provide an opportunity for public and Federal agency comment. Specifically, the agenda of the RAC meeting citing the major actions will be published in the Federal Register at least 30 days before the meeting, and the Director will also publish the proposed actions in the Federal Register for comment at least 30 days before the meeting. In addition, the Director's proposed decision, at his discretion, may be published in the Federal Register for 30 days of comment before final action is taken.

The Director's final decision, along with response to the comments, will be published in the Federal Register and the Recombinant DNA Technical Bulletin. The RAC and IBC chairpersons will be notified of this decision:

IV-C-1-b-(1)-(a). Changing containment levels for types of experiments that are specified in the Guidelines when a major action is involved;

IV-C-1-b-(1)-(b). Assigning containment levels for types of experiments that are not explicitly considered in the Guidelines when a major action is involved;

IV-C-1-b-(1)-(c). Promulgating and amending a list of classes of recombinant DNA molecules to be exempt from these Guidelines because they consist entirely of DNA segments from species that exchange DNA by known physiological processes, or otherwise do not present a significant risk to health or the environment;

IV-C-1-b-(1)-(d). Permitting experiments specified by Section III-A of the Guidelines;

IV-C-1-b-(1)-(e). Certifying new host-vector systems, with the exception of minor modifications of already certified systems (the standards and procedures for certification are described in Appendix I-II-A. Minor modifications constitute, for example, those of minimal or no consequence to the properties relevant to containment); and

IV-C-1-b-(1)-(f). Adopting other changes in the Guidelines.

IV-C-1-b-(2). Lesser Actions. To execute lesser actions, the Director must seek the advice of the RAC. The Director's decision will be transmitted to the RAC and IBC chairpersons and published in the Recombinant DNA Technical Bulletin:

IV-C-1-b-(2)-(a). Interpreting and determining containment levels, upon request by ORDA;

IV-C-1-b-(2)-(b). Changing containment levels for experiments that are specified in the Guidelines (see Section III);

IV-C-1-b-(2)-(c). Assigning containment levels for experiments not explicitly considered in the Guidelines;

IV-C-1-b-(2)-(d). Revising the "Classification of Etiologic Agents" for the purpose of these Guidelines [1].

IV-C-1-b-(3). Other Actions. The Director's decision will be transmitted to the RAC and IBC chairpersons and published in the Recombinant DNA Technical Bulletin:

IV-C-1-b-(3)-(a). Interpreting the Guidelines for experiments to which the Guidelines specifically assign containment levels;

IV-C-1-b-(3)-(b). Setting containment under Section III-B-1-d and Section III-B-3-d;

IV-C-1-b-(3)-(c). Approving minor modifications of already certified host-vector systems (the standards and procedures for such modifications are described in Appendix I-II);

IV-C-1-b-(3)-(d). Decertifying already certified host-vector systems;

IV-C-1-b-(3)-(e). Adding new entries to the list of molecules toxic for vertebrates (see Appendix F);

IV-C-1-b-(3)-(f). Approving the cloning of toxin genes in host-vector systems other than E. coli K-12 (See Appendix F); and

IV-C-1-b-(3)-(g). Determining appropriate containment conditions for experiments according to case precedents developed under Section IV-C-1-b-(2)-(c).

IV-C-1-b-(4). The Director shall conduct, support, and assist training programs in laboratory safety for Institutional Biosafety Committee members, Biological Safety Officers, Principal Investigators, and laboratory staff.

IV-C-2. Recombinant DNA Advisory Committee. The NIH Recombinant DNA Advisory Committee (RAC) is responsible for carrying out specified functions cited below as well as others assigned under its charter or by the Secretary, HHS, the Assistant Secretary for Health, and the Director, NIH.

The members of the committee shall be chosen to provide, collectively, expertise in scientific fields relevant to recombinant DNA technology and biological safety—e.g., microbiology, molecular biology, virology, genetics,

epidemiology, infectious diseases, the biology of enteric organisms, botany, plant pathology, ecology, and tissue culture. At least 20 percent of the members shall be persons knowledgeable in applicable law, standards of professional conduct and practice, public attitudes, the environment, public health, occupational health, or related fields. Representatives from Federal agencies shall serve as nonvoting members. Nominations for the RAC may be submitted to the NIH Office of Recombinant DNA Activities, Bethesda, Md. 20205.

All meetings of the RAC will be announced in the Federal Register, including tentative agenda items, 30 days in advance of the meeting, with final agendas (if modified) available at least 72 hours before the meeting. No item defined as a major action under Section IV-C-1-b-(1) may be added to an agenda after it appears in the Federal Register.

The RAC shall be responsible for advising the Director, NIH, on the actions listed in Section IV-C-1-b-(1) and IV-C-1-b-(2).

IV-C-3. The Office of Recombinant DNA Activities. ORDA shall serve as a focal point for information on recombinant DNA activities and provide advice to all within and outside NIH, including Institutions, Biological Safety Committees, Principal Investigators, Federal agencies, State and local governments, and institutions in the private sector. ORDA shall carry out such other functions as may be delegated to it by the Director, NIH, including

those authorities described in Section IV-C-1-b-(3). In addition, ORDA shall be responsible for the following:

IV-C-3-a. Reviewing and approving Institutional Biosafety Committee (IBC) membership;

IV-C-3-b. Publishing in the Federal Register:

IV-C-3-b-(1). Announcements of Recombinant DNA Advisory Committee (RAC) meetings and agendas at least 30 days in advance;

NOTE: If the agenda for an RAC meeting is modified, ORDA shall make the revised agenda available to anyone, upon request, at least 72 hours in advance of the meeting.

IV-C-3-b-(2). Proposed major actions of the type falling under Section IV-C-1-b-(1) at least 30 days prior to the RAC meeting at which they will be considered; and

IV-C-3-b-(3). The NIH Director's final decision on recommendations made by the RAC.

IV-C-3-c. Publishing the Recombinant DNA Technical Bulletin; and

IV-C-3-d. Serving as executive secretary of the RAC.

IV-C-4. Other NIH Components. Other NIH components shall be responsible for certifying P4 facilities, inspecting them periodically, and inspecting other recombinant DNA facilities as deemed necessary.

IV-D. Compliance. As a condition for NIH funding of recombinant DNA research, Institutions must ensure that such research conducted at or sponsored by the Institution, irrespective of the source of funding, shall comply with these Guidelines. The policies on noncompliance are as follows:

IV-D-1. All NIH-funded projects involving recombinant DNA techniques must comply with the NIH Guidelines. Noncompliance may result in (i) suspension, limitation, or termination of financial assistance for such projects and of NIH funds for other recombinant DNA research at the Institution, or (ii) a requirement for prior NIH approval of any or all recombinant DNA projects at the Institution.

IV-D-2. All non-NIH funded projects involving recombinant DNA techniques conducted at or sponsored by an Institution that receives NIH funds for projects involving such techniques must comply with the NIH Guidelines. Noncompliance may result in (i) suspension, limitation, or termination of NIH funds for recombinant DNA research at the Institution, or (ii) a requirement for prior NIH approval of any or all recombinant DNA projects at the Institution.

IV-D-3. Information concerning noncompliance with the Guidelines may be brought forward by any person. It should be delivered to both NIH (ORDA) and the relevant Institution. The Institution, generally through the IBC, shall take appropriate action. The Institution shall forward a complete report of the incident to ORDA, recommending any further action indicated.

IV-D-4. In cases where NIH proposes to suspend, limit, or terminate financial assistance because of noncompliance with the Guidelines, applicable DHHS and Public Health Service procedures shall govern.

See Appendix J for information on the Federal Interagency Advisory Committee on Recombinant DNA Research.

IV-D-5. Voluntary Compliance. Any individual, corporation, or institution that is not otherwise covered by the Guidelines is encouraged to conduct recombinant DNA research activities in accordance with the Guidelines, through the procedures set forth in Part VI.

V. FOOTNOTES AND REFERENCES

OF SECTIONS I-IV

1. The original reference to organisms as Class 1, 2, 3, 4, or 5 refers to the classification in the publication Classification of Etiologic Agents on the Basis of Hazard, 4th Edition, July 1974; U.S. Department of Health, Education, and Welfare, Public Health Service, Centers for Disease Control, Office of Biosafety, Atlanta, Georgia 30333.

   The Director, NIH, with advice of the Recombinant DNA Advisory Committee, may revise the classification for the purposes of these Guidelines (see Section IV-C-1-b-(2)-(d)). The revised list of organisms in each class is reprinted in Appendix B to these Guidelines.

2. In Part III of the Guidelines, there are a number of places where judgments are to be made. In all these cases the principal investigator is to make the judgment on these matters as part of his responsibility to "make the initial determination of the required levels of physical and biological containment in accordance with the Guidelines" (Section IV-B-5-c-(1)). In the cases falling under Sections III-A, -B or -C, this judgment is to be reviewed and approved by the Institutional Biosafety Committee as part of its responsibility to make "an independent assessment of the containment levels required by these Guidelines for the proposed research" (Section IV-B-3-a-(1)). If the IBC wishes, any specific cases may be referred to the NIH Office of Recombinant DNA Activities as part of ORDA's functions to "provide advice to all within and outside NIH" (Section IV-C-3), and ORDA may request advice from the Recombinant DNA Advisory Committee as part of the RAC's responsibility for "interpreting and determining containment levels upon request by ORDA" (Section IV-C-1-b-(2)-(a)).

3. Laboratory Safety at the Center for Disease Control (Sept. 1974). U.S. Department of Health Education and Welfare Publication No. CDC 75-8118.

4. Classification of Etiologic Agents on the Basis of Hazard. (4th Edition, July 1974). U.S. Department of Health, Education and Welfare. Public Health Service. Centers for Disease Control, Office of Biosafety, Atlanta, Georgia 30333.

5. National Cancer Institute Safety Standards for Research Involving Oncogenic Viruses (Oct. 1974). U.S. Department of Health, Education and Welfare Publication No. (NIH) 75-790.

6. National Institutes of Health Biohazards Safety Guide (1974). U.S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health. U.S. Government Printing Office, Stock No. 1740-00383.

7. Biohazards in Biological Research (1973). A. Hellman, M. N. Oxman, and R. Pollack (ed.) Cold Spring Harbor Laboratory.

8. Handbook of Laboratory Safety (1971). Second Edition. N. V. Steere (ed.). The Chemical Rubber Co., Cleveland.

9. Bodily, J. L. (1970). General Administration of the Laboratory, H. L. Bodily, E. L. Updyke, and J. O. Mason (eds.), Diagnostic Procedures for Bacterial, Mycotic and Parasitic Infections. American Public Health Association, New York, pp. 11-28.

10. Darlow, H. M. (1969). Safety in the Microbiological Laboratory. In J. R. Norris and D. W. Robbins (ed.), Methods in Microbiology. Academic Press, Inc. New York pp. 169-204.

11. The Prevention of Laboratory Acquired Infection (1974). C. H. Collins, E. G. Hartley, and R. Pilsworth. Public Health Laboratory Service, Monograph Series No. 6.

12. Chatigny, M. A. (1961). Protection Against Infection in the Microbiological Laboratory: Devices and Procedures. In W. W. Umbreit (ed.). Advances in Applied Microbiology. Academic Press, New York, N.Y. 3:131-192.

13. Design Criteria for Viral Oncology Research Facilities (1975). U.S. Department of Health, Education and Welfare, Public Health Service, National Institutes of Health, DHEW Publication No. (NIH) 75-891.

14. Kuehne, R. W. (1973). Biological Containment Facility for Studying Infectious Disease. Appl. Microbiol. 26-239-243.

15. Runkle, R. S., and G. B. Phillips (1969). Microbial Containment Control Facilities. Van Nostrand Reinhold, New York.

16. Chatigny, M. A., and D. I. Clinger (1969). Contamination Control in Aerobiology. In R. L. Dimmick and A. B. Akers (eds.). An Introduction to Experimental Aerobiology. John Wiley & Sons, New York, pp. 194-263.

17. As classified in the Third Report of the International Committee on Taxonomy of Viruses: Classification and Nomenclature of Viruses, R. E. F. Matthews, Ed. Intervirology 12 (129-296) 1979.

18. A USDA permit, required for import and interstate transport of pathogens, may be obtained from the Animal and Plant Health Inspection Service, USDA, Federal Building, Hyattsville, MD 20782.

19. i.e., the total of all genomes within a Family shall not exceed two-thirds of the genome.

20. All activities, including storage of variola and whitepox are restricted to the single national facility (World Health Organization (WHO) Collaborating Center for Smallpox Research, Centers for Disease Control, in Atlanta).

VI. VOLUNTARY COMPLIANCE

VI-A. Basic Policy. Individuals, corporations, and institutions not otherwise covered by the Guidelines are encouraged to do so by following the standards and procedures set forth in Parts I-IV of the Guidelines. In order to simplify discussion, references hereafter to "institutions" are intended to encompass corporations, and individuals who have no organizational affiliation. For purposes of complying with the Guidelines, an individual intending to carry out research involving recombinant DNA is encouraged to affiliate with an institution that has an Institutional Biosafety Committee approved under the Guidelines.

Since commercial organizations have special concerns, such as protection of proprietary data, some modifications and explanations of the procedures in Parts I-IV are provided below, in order to address these concerns.

VI-B. IBC Approval. The NIH Office of Recombinant DNA Activities (ORDA) will review the membership of an institution's Institutional Biosafety Committee (IBC) and, where it finds the IBC meets the requirements set forth in Section IV-B-2, will give its approval to the IBC membership.

It should be emphasized that employment of an IBC member solely for purposes of membership on the IBC does not itself make the member an institutionally affiliated member for purposes of Section IV-B-2-a.

Except for the unaffiliated members, a member of an IBC for an institution not otherwise covered by the Guidelines may participate in the review and approval of a project in which the member has a direct financial interest, so long as the member has not been and does not expect to be engaged in the project. Section IV-B-2-d is modified to that extent for purposes of these institutions.

VI-C. Certification of Host-Vector Systems. A host-vector system may be proposed for certification by the Director, NIH, in accordance with the procedures set forth in Appendix I-II-A.

In order to ensure protection for proprietary data, any public notice regarding a host-vector system which is designated by the institution as proprietary under Section VI-E-1 will be issued only after consultation with the institution as to the content of the notice.

VI-D. Requests for Exemptions and Approvals. Requests for exemptions or other approvals required by the Guidelines should be requested by following the procedures set forth in the appropriate sections in Parts I-IV of the Guidelines.

In order to ensure protection for proprietary data, any public notice regarding a request for an exemption or other approval which is designated by the institution as proprietary under Section VI-E-1 will be issued only after consultation with the institution as to the content of the notice.

VI-E. Protection of Proprietary Data. In general, the Freedom of Information Act requires Federal agencies to make their records available to the public upon request. However, this requirement does not apply to, among other things, "trade secrets and commercial and financial information obtained from a person and privileged or confidential." 18 U.S.C. 1905, in turn makes it a crime for an officer or employee of the United States or any Federal department or agency to publish, divulge, disclose, or make known "in any manner or to any extent not authorized by law any information coming to him in the course of his employment or official duties or by reason of any examination or investigation made by, or return, report or record made to or filed with, such department or agency or officer or employee thereof, which information concerns or relates to the trade secrets, [or processes...of any person, firm, partnership, corporation, or association." This provision applies to all employees of the Federal Government, including special Government employees. Members of the Recombinant DNA Advisory Committee are "special Government employees."

VI-E-1. In submitting information to NIH for purposes of complying voluntarily with the Guidelines, an institution may designate those items of information which the institution believes constitute trade secrets or privileged or confidential commercial or financial information.

VI-E-2. If NIH receives a request under the Freedom of Information Act for information so designated, NIH will promptly contact the institution to secure its views as to whether the information (or some portion) should be released.

VI-E-3. If the NIH decides to release this information (or some portion) in response to a Freedom of Information request or otherwise, the institution will be advised; and the actual release will not be made until the expiration of 15 days after the institution is so advised, except to the extent that earlier release, in the judgement of the Director, NIH, is necessary to protect against an imminent hazard to the public or the environment.

VI-E-4. Presubmission Review.

VI-E-4-a. Any institution not otherwise covered by the Guidelines, which is considering submission of data or information voluntarily to NIH, may request presubmission review of the records involved to determine whether, if the records are submitted, NIH will or will not make part or all of the records available upon request under the Freedom of Information Act.

VI-E-4-b. A request for presubmission review should be submitted to ORDA, along with the records involved. These records must be clearly marked as being the property of the institution, on loan to NIH solely for the purpose of making a determination under the Freedom of Information Act. ORDA will then seek a determination from the HHS Freedom of Information Officer, the responsible official under HHS regulations (45 C.F.R. Part 5), as to whether the records involved (or some portion) are or are not available

to members of the public under the Freedom of Information Act. Pending such a determination, the records will be kept separate from ORDA files, will be considered records of the institution and not ORDA, and will not be received as part of ORDA files. No copies will be made of the records.

VI-E-4-c. ORDA will inform the institution of the HHS Freedom of Information Officer's determination and follow the institution's instructions as to whether some or all of the records involved are to be returned to the institution or to become a part of ORDA files. If the institution instructs ORDA to return the records, no copies or summaries of the records will be made or retained by HHS, NIH, or ORDA.

VI-E-4-d. The HHS Freedom of Information Officer's determination will represent that official's judgement, as of the time of the determination, as to whether the records involved (or some portion) would be exempt from disclosure under the Freedom of Information Act, if at the time of the determination the records were in ORDA files and a request were received from them under the Act.

APPENDIX A

EXEMPTIONS UNDER III-D-4

Section III-D-4 states that exempt from these Guidelines are "certain specified recombinant DNA molecules that consist entirely of DNA segments from different species that exchange DNA by known physiological processes, though one or more of the segments may be a synthetic equivalent. A list of such exchangers will be prepared and periodically revised by the Director, NIH, with advice of the RAC, after appropriate notice and opportunity for public comment. (See Section IV-C-1-b-(1)-(c).) Certain classes are exempt as of publication of these Revised Guidelines. The list is in Appendix A."

Under Section III-D-4 of these Guidelines are recombinant DNA molecules that are (1) composed entirely of DNA segments from one or more of the organisms within a sublist and (2) to be propagated in any of the organisms within a sublist. (Classification of Bergey's Manual of Determinative Bacteriology, eighth edition. R. E. Buchanan and N. E. Gibbons, editors. Williams and Wilkins Company: Baltimore, 1974.)

Sublist A

1. Genus Escherichia
2. Genus Shigella
3. Genus Salmonella (including Arizona)
4. Genus Enterobacter
5. Genus Citrobacter (including Levinea)
6. Genus Klebsiella
7. Genus Erwinia

8. Pseudomonas aeruginosa, Pseudomonas putida and Pseudomonas fluorescens
9. Serratia marcescens
10. Yersinia enterocolitica

Sublist B

1. Bacillus subtilis
2. Bacillus licheniformis
3. Bacillus pumilus
4. Bacillus globigii
5. Bacillus niger
6. Bacillus nato
7. Bacillus amyloliquefaciens
8. Bacillus aterrimus

Sublist C

1. Streptomyces aureofaciens
2. Streptomyces rimosus
3. Streptomyces coelicolor

Sublist D

1. Streptomyces griseus
2. Streptomyces cyaneus
3. Streptomyces venezuelae

Sublist E

One way transfer of Streptococcus mutans or Streptococcus lactis DNA into Streptococcus sanguis.

Sublist F

1. Streptococcus sanguis
2. Streptococcus pneumoniae
3. Streptococcus faecalis
4. Streptococcus pyogenes

APPENDIX B

CLASSIFICATION OF MICROORGANISMS ON THE BASIS OF HAZARD

Appendix B-I. Classification of Etiologic Agents. [The original reference for this classification was the publication "Classification of Etiological Agents on the Basis of Hazard," 4th edition, July 1974, U.S. Department of Health, Education, and Welfare, Public Health Service, Center for Disease Control, Office of Biosafety, Atlanta, Georgia 30333. For the purposes of these Guidelines, this list has been revised by the NIH.]

Appendix B-I-A. Class 1 Agents. All bacterial, parasitic, fungal, viral, rickettsial, and chlamydial agents not included in higher classes.

Appendix B-I-B. Class 2 Agents.

Appendix B-I-B-1. Bacterial Agents.

Acinetobacter calcoaceticus
Actinobacillus - all species
Aeromonas hydrophila
Arizona hinshawii - all serotypes
Bacillus anthracis
Bordetella - all species
Borrelia recurrentis, B. vincenti
Campylobacter fetus
Campylobacter jejuni
Chlamydia psittaci
Chlamydia trachomatis
Clostridium botulinum,
Cl. chauvoei, Cl. haemolyticum,
Cl. histolyticum, Cl. novyi,
Cl. septicum, Cl. tetani
Corynebacterium diphtheriae,
C. equi, C. haemolyticum,
C. pseudotuberculosis,
C. pyogenes, C. renale

Edwardsiella tarda
Erysipelothrix insidiosa
Escherichia coli - all enteropathogenic, enterotoxigenic, enteroinvasive and strains bearing K1 antigen
Haemophilus ducreyi, H. influenzae
Klebsiella - all species and all serotypes
Legionella pneumophila
Leptospira interrogans - all serotypes
Listeria - all species
Moraxella - all species
Mycobacteria - all species except those listed in Class 3
Mycoplasma - all species except Mycoplasma mycoides and Mycoplasma agalactiae, which are in Class 5
Neisseria gonorrhoeae, N. meningitidis
Pasteurella - all species except those listed in Class 3
Salmonella - all species and all serotypes
Shigella - all species and all serotypes
Sphaerophorus necrophorus
Staphylococcus aureus
Streptobacillus moniliformis
Streptococcus pneumoniae
Streptococcus pyogenes
Treponema carateum, T. pallidum, and T. pertenue
Vibrio cholerae
Vibrio parahemolyticus
Yersinia enterocolitica

Appendix B-I-B-2. Fungal Agents.

Actinomycetes (including Nocardia species and Actinomyces species and Arachnia propionica) [2]
Blastomyces dermatitidis
Cryptococcus neoformans
Paracoccidioides braziliensis

Appendix B-I-B-3. Parasitic Agents.

Endamoeba histolytica
Leishmania sp.
Naegleria gruberi
Schistosoma mansoni
Toxoplasma gondii
Toxocara canis
Trichinella spiralis
Trypanosoma cruzi

Appendix B-I-B-4. Viral, Rickettsial, and Chlamydial Agents.

Adenoviruses - human - all types
Cache Valley virus
Coxsackie A and B viruses
Cytomegaloviruses
Echoviruses - all types
Encephalomyocarditis virus (EMC)
Flanders virus
Hart Park virus
Hepatitis-associated antigen material
Herpes viruses - except Herpesvirus simiae (Monkey B virus) which is in Class 4
Corona viruses
Influenza viruses - all types except A/PR8/34, which is in Class 1
Langat virus
Lymphogranuloma venereum agent
Measles virus
Mumps virus
Parainfluenza virus - all types except Parainfluenza virus 3, SF4 strain, which is in Class 1
Polioviruses - all types, wild and attenuated
Poxviruses - all types except Alastrim, Smallpox, and Whitepox, which are Class 5 and Monkey pox, which depending on experiments, is in Class 3 or Class 4
Rabies virus - all strains except Rabies street virus, which should be classified in Class 3
Reoviruses - all types
Respiratory syncytial virus
Rhinoviruses - all types
Rubella virus

Simian viruses - all types except Herpesvirus simiae (Monkey B virus) and Marburg virus, which are in Class 4
Sindbis virus
Tensaw virus
Turlock virus
Vaccinia virus
Varicella virus
Vesicular stomatitis virus [3]
Vole rickettsia
Yellow fever virus, 17D vaccine strain

Appendix B-I-C. Class 3 Agents.

Appendix B-I-C-1. Bacterial Agents.

Bartonella - all species
Brucella - all species
Francisella tularensis
Mycobacterium avium, M. bovis, M. tuberculosis
Pasteurella multocide type B ("buffalo" and other foreign virulent strains) [3]
Pseudomonas mallei [3]
Pseudomonas pseudomallei [3]
Yersinia pestis

Appendix B-I-C-2. Fungal Agents.

Coccidioides immitis
Histoplasma capsulatum
Histoplasma capsulatum var. duboisii

Appendix B-I-C-3. Parasitic Agents.

None.

Appendix B-I-C-4. Viral, Rickettsial, and Chlamydial Agents.

Monkey pox, when used in vitro [4]
Arboviruses - all strains except those in Class 2 and 4 (Arboviruses indigenous to the United States are in Class 3, except those listed in Class 2. West Nile and Semliki Forest viruses may be classified up or down, depending on the conditions of use and geographical location of the laboratory.)
Dengue virus, when used for transmission or animal inoculation experiments
Lymphocytic choriomeningitis virus (LCM)
Rabies street virus
Rickettsia - all species except Vole rickettsia when used for transmission or animal inoculation experiments
Yellow fever virus - wild, when used in vitro

Appendix B-I-D. Class 4 Agents.

Appendix B-I-D-1. Bacterial Agents.

None.

Appendix B-I-D-2. Fungal Agents.

None.

Appendix B-I-D-3. Parasitic Agents.

None.

Appendix B-I-D-4. Viral, Rickettsial, and Chlamydial Agents.

Ebola fever virus
Monkey pox, when used for transmission or animal inoculation experiments [4]

Hemorrhagic fever agents, including Crimean hemorrhagic fever, (Congo), Junin, and Machupo viruses, and others as yet undefined
Herpesvirus simiae (Monkey B virus)
Lassa virus
Marburg virus
Tick-borne encephalitis virus complex, including Russian spring-summer encephalitis, Kyasanur forest disease, Omsk hemorrhagic fever, and Central European encephalitis viruses
Venezuelan equine encephalitis virus, epidemic strains, when used for transmission or animal inoculation experiments
Yellow fever virus - wild, when used for transmission or animal inoculation experiments

Appendix B-II. Classification of Oncogenic Viruses on the Basis of Potential Hazard [5].

Appendix B-II-A. Low-Risk Oncogenic Viruses.

Rous Sarcoma
SV-40
CELO
Ad7-SV40
Polyoma
Bovine papilloma
Rat mammary tumor
Avian Leukosis
Murine Leukemia
Murine Sarcoma
Mouse mammary tumor
Rat Leukemia
Hamster Leukemia
Bovine Leukemia
Dog Sarcoma
Mason-Pfizer Monkey Virus
Marek's
Guinea Pig Herpes
Lucke (Frog)
Adenovirus
Shope Fibroma
Shope Papilloma

Appendix B-II-B. Moderate-Risk Oncogenic Viruses.

Ad2-SV40
FeLV
HV Saimiri
EBV
SSV-1
GaLV
HV ateles
Yaba
FeSV

Appendix B-III. Class 5 Agents.

Appendix B-III-A. Animal Disease Organisms Which are Forbidden Entry into the United States by Law.

Foot and mouth disease virus.

Appendix B-III-B. Animal Disease Organisms and Vectors Which are Forbidden Entry into the United States by USDA Policy.

African horse sickness virus
African swine fever virus
Besnoitia besnoiti
Borna disease virus
Bovine infectious petechial fever
Camel pox virus
Ephemeral fever virus

Fowl plague virus
Goat pox virus
Hog cholera virus
Louping ill virus
Lumpy skin disease virus
Nairobi sheep disease virus
Newcastle disease virus (Asiatic strains)
Mycoplasma mycoides (contagious bovine pleuropneumonia)
Mycoplasma agalactiae (contagious agalactia of sheep)
Rickettsia ruminatium (heart water)
Rift valley fever virus
Rhinderpest virus
Sheep pox virus
Swine vesicular disease virus
Teschen disease virus
Trypanosoma vivax (Nagana)
Trypanosoma evansi
Theileria parva (East Coast fever)
Theileria annulata
Theileria lawrencei
Theileria bovis
Theileria hirci
Vesicular exanthema virus
Wesselsbron disease virus
Zyonema

Appendix B-III-C. Organisms Which May Not Be Studied in the United States Except At Specified Facilities.

Small pox [4]
Alastrim [4]
White pox [4]

Appendix B-IV. Footnotes and References of Appendix B.

1. The original reference for this classification was the publication Classification of Etiologic Agents on the Basis of Hazard, 4th edition, July 1974, U.S. Department of Health, Education, and Welfare, Public Health Service, Center for Disease Control, Office of Biosafety, Atlanta, Georgia 30333. For the purposes of these Guidelines, this list has been revised by the NIH.

2. Since the publication of the classification in 1974 [1], the Actinomycetes have been reclassified as bacterial rather than fungal agents.

3. A USDA permit, required for import and interstate transport of pathogens, may be obtained from the Animal and Plant Health Inspection Service, USDA, Federal Building, Hyattsville, MD 20782.

4. All activities, including storage of variola and whitepox are restricted to the single national facility [World Health Organization (WHO) Collaborating Center for Smallpox Research, Center for Disease Control, in Atlanta].

5. National Cancer Institute Safety Standards for Research Involving Oncogenic Viruses (October 1974). U.S. Department of Health, Education, and Welfare Publication No. (NIH) 75-790.

6. U.S. Department of Agriculture, Animal and Plant Health Inspection Service.

APPENDIX C

EXEMPTIONS UNDER III-D-5

Section III-D-5 states that exempt from these Guidelines are "Other classes of recombinant DNA molecules, if the Director, NIH, with advice of the RAC, after appropriate notice and opportunity for public comment, finds that they do not present a significant risk to health or the environment. (See Section IV-C-1-b-(1)-(c).) Certain classes are exempt as of publication of these Revised Guidelines."

The following classes of experiments are exempt under Section III-D-5 of the Guidelines:

Appendix C-I. Recombinant DNAs in Tissue Culture. Recombinant DNA molecules derived entirely from non-viral components (that is, no component is derived from a eukaryotic virus), that are propagated and maintained in cells in tissue culture are exempt from these Guidelines with the exceptions listed below.

Exceptions.

Experiments described in Section III-A which require specific RAC review and NIH approval before initiation of the experiment.

Experiments involving DNA from Class 3, 4, or 5 organisms [1] or cells known to be infected with these agents.

Experiments involving the deliberate introduction of genes coding for the biosynthesis of molecules toxic for vertebrates. (See Appendix F.)

Appendix C-II. Experiments Involving E. coli K-12 Host-Vector Systems. Experiments which use E. coli K-12 host-vector systems, with the exception of those experiments listed below, are exempt from these Guidelines provided that (a) the E. coli host shall not contain conjugation proficient plasmids or generalized transducing phages, and (b) lambda or lambdoid or Ff bacteriophages or nonconjugative plasmids [2] shall be used as vectors. However, experiments involving the insertion into E. coli K-12 of DNA from prokaryotes that exchange genetic information [3] with E. coli may be performed with any E. coli K-12 vector (e.g., conjugative plasmid). When a nonconjugative vector is used, the E. coli K-12 host may contain conjugation-proficient plasmids either autonomous or integrated, or generalized transducing phages.

For these exempt experiments, P1 physical containment conditions are recommended.

Exceptions

Experiments described in Section III-A which require specific RAC review and NIH approval before initiation of the experiment.

Experiments involving DNA from Class 3, 4, or 5 organisms [1] or from cells known to be infected with these agents may be conducted under containment conditions specified in Section III-B-2 with prior IBC review and approval.

Large-scale experiments (e.g., more than 10 liters of culture) require prior IBC review and approval. (See Section III-B-5.)

Experiments involving the deliberate cloning of genes coding for the biosynthesis of molecules toxic for vertebrates. (See Appendix F.)

Appendix C-III. Experiments Involving Saccharomyces cerevisiae Host-Vector Systems. Experiments which use Saccharomyces cerevisiae host-vector systems, with the exception of experiments listed below, are exempt from these Guidelines provided that laboratory strains are used.

For these exempt experiments, P1 physical containment conditions are recommended.

Exceptions

Experiments described in Section III-A which require specific RAC review and NIH approval before initiation of the experiment.

Experiments involving Class 3, 4 or 5 organisms [1] or cells known to be infected with these agents may be conducted under containment conditions specified in Section III-B-2 with prior IBC review and approval.

Large-scale experiments (e.g., more than 10 liters of culture) require prior IBC review and approval. (See Section III-B-5.)

Experiments involving the deliberate cloning of genes coding for the biosynthesis of molecules toxic for vertebrates. (See Appendix F.)

Appendix C-IV. Experiments Involving Bacillus subtilis Host-Vector Systems. Any asporogenic Bacillus subtilis strain which does not revert to a sporeformer with a frequency greater than 10^{-7} can be used for cloning DNA, with the exception of those experiments listed below. Indigenous Bacillus plasmids and phages, whose host-range does not include Bacillus cereus or Bacillus anthracis, may be used as vectors.

For these exempt experiments P1 physical containment conditions are recommended.

Exceptions

Experiments described in Section III-A which require specific RAC review and approval before initiation of the experiment.

Experiments involving Class 3, 4, or 5 organisms [1] or cells known to be infected with these agents may be conducted under containment conditions specified by Section III-B-2 with prior IBC review and approval.

Large-scale experiments (e.g., more than 10 liters of culture) require prior IBC review and approval. (See Section III-B-5.)

Experiments involving the deliberate cloning of genes coding for the biosynthesis of molecules toxic for vertebrates. (See Appendix F.)

Appendix C-V. Footnotes and References of Appendix C.

1. The original reference to organisms as Class 1, 2, 3, 4, or 5 refers to the classification in the publication Classification of Etiologic Agents on the Basis of Hazard, 4th Edition, July 1974; U.S. Department of Health, Education, and Welfare, Public Health Service, Centers for Disease Control, Office of Biosafety, Atlanta, Georgia 30333.

   The Director, NIH, with advice of the Recombinant DNA Advisory Committee, may revise the classification for the purposes of these Guidelines (see Section IV-C-1-b-(2)-(d)). The revised list of organisms in each class is reprinted in Appendix B to these Guidelines.

2. A subset of non-conjugative plasmid vectors are also poorly mobilizable (e.g., pBR322, pBR313). Where practical, these vectors should be employed.

3. Defined as observable under optimal laboratory conditions by transformation, transduction, phage infection, and/or conjugation with transfer of phage, plasmid, and/or chromosomal genetic information. Note that this definition of exchange may be less stringent than that applied to exempt organisms under Section III-D-4.

APPENDIX D

ACTIONS TAKEN UNDER THE GUIDELINES

As noted in the subsections of Section IV-C-1-b-(1), the Director, NIH, may take certain actions with regard to the Guidelines after the issues have been considered by the RAC

Some of the actions taken to date include the following:

Appendix D-I. Permission is granted to clone Foot-and-Mouth Disease Virus in the EK1 host-vector system consisting of E. coli K-12 and the vector pBR322, all work to be done at the Plum Island Animal Disease Center.

Appendix D-II. Certain specified clones derived from segments of the Foot-and-Mouth Disease Virus may be transferred from Plum Island Animal Disease Center to the facilities of Genentech, Inc., of South San Francisco, California. Further development of the clones at Genentech has been approved under P1 + EK1 conditions.

Appendix D-III. The Rd strain of Hemophilus influenzae can be used as a host for the propagation of the cloned Tn 10 tet R gene derived from E. coli K-12 employing the non-conjugative Haemophilus plasmid, pRSF0885, under P1 conditions.

Appendix D-IV. Permission is granted to clone certain subgenomic segments of Foot-and-Mouth Disease Virus in HV1 Bacillus subtilis and Saccharomyces cerevisiae host-vector systems under P1 conditions at Genentech, Inc., South San Francisco, California.

Appendix D-V. Permission is granted to Dr. Ronald Davis of Stanford University to field test corn plants modified by recombinant DNA techniques under specified containment conditions.

Appendix D-VI. Permission is granted to clone in E. coli K-12, under P1 physical containment conditions, subgenomic segments of Rift Valley Fever Virus subject to conditions which have been set forth by the RAC.

Appendix D-VII. Attenuated laboratory strains of Salmonella typhimurium may be used under P1 physical containment conditions to screen for the Saccharomyces cerevisiae pseudouridine synthetase gene. The plasmid YEp13 will be employed as the vector.

Appendix D-VIII. Permission is granted to transfer certain clones of subgenomic segments of Foot-and-Mouth Disease Virus from Plum Island Animal Disease Center to the laboratories of Molecular Genetics, Inc., Minnetonka, Minnesota, and to work with these clones under P1 containment conditions. Approval is contingent upon review of data on infectivity testing of the clones by a working group of the RAC.

APPENDIX E

CERTIFIED HOST-VECTOR SYSTEMS

While many experiments using E. coli K-12, Saccharomyces cerevisiae and Bacillus subtilis are currently exempt from the Guidelines under Exemption III-D-5, some derivatives of these host-vector systems were previously classified as HV1 or HV2. A listing of those systems follows.

HV1. The following plasmids are accepted as the vector components of certified B. subtilis HV1 systems: pUB110, pC194, pS194, pSA2100, pE194, pT127, pUB112, pC221, pC223, and pAB124. B. subtilis strains RUB 331 and BGSC 1S53 have been certified as the host component of HV1 systems based on these plasmids.

HV2. The asporogenic mutant derivative of Bacillus subtilis, ASB 298, with the following plasmids as the vector component: pUB110, pC194, pS194, pSA2100, pE194, pT127, pUB112, pC221, pC223, and pAB124.

HV2 - The following sterile strains of Saccharomyces cerevisiae, all of which have the ste-VC9 mutation, SHY1, SHY2, SHY3, and SHY4. The following plasmids are certified for use: YIp1, YEp2, YEp4, YIp5, YEp6, YRp7, YEp20, YEp21, YEp24, YIp25, YIp26, YIp27, YIp28, YIp29, YIp30, YIp31, YIp32, and YIp33.

EK2 Plasmid Systems. The E. coli K-12 strain chi-1776. The following plasmids are certified for use: pSC101, pMB9, pBR313, pBR322, pDH24, pBR325, pBR327, pGL101, pHB1. The following E. coli/S. cerevisiae hybrid plasmids are certified as EK2 vectors when used in E. coli chi-1776 or in the sterile yeast strains, SHY1, SHY2, SHY3 and SHY4: YIp1, YEp2, YEp4, YIp5, YEp6, YRp7, YEp20, YEp21, YEp24, YIp25, YIp26, YIp27, YIp28, YIp29, YIp30, YIp31, YIp32, YIp33.

EK2 Bacteriophage Systems. The following are certified EK2 systems based on bacteriophage lambda:

Vector	Host
λgtWES.λB'	DP50supF
λgtWES.λB*	DP50supF
λgtZJvir.λB'	E. coli K-12
λgtALO.λB	DP50supF
Charon 3A	DP50 or DP50supF
Charon 4A	DP50 or DP50supF
Charon 16A	DP50 or DP50supF
Charon 21A	DP50supF
Charon 23A	DP50 or DP50supF
Charon 24A	DP50 or DP50supF

E. coli K-12 strains chi-2447 and chi-2281 are certified for use with lambda vectors that are certified for use with strain DP50 or DP50supF provided that the su⁻ strain not be used as a propagation host.

Additional certified host-vector systems.

HV1 - The following specified strains of Neurospora crassa which have been modified to prevent aerial dispersion:

In1 (inositolless) strains 37102, 37401, 46316, 64001, and 89601.

Csp-1 strain UCLA37 and csp-2 strains FS 590, UCLA101 (these are conidial separation mutants).

Eas strain UCLA191 (an "easily wettable" mutant).

HV1- The following Streptomyces species: Streptomyces coelicolor, S. lividans, S. parvulus, and S. griseus. The following are accepted as vector components of certified Streptomyces HV1 systems: Streptomyces plasmids SCP2, SLP1.2, pLJ101, actinophage phi C31, and their derivatives.

HV1- Pseudomonas putida strain KT2440 with plasmid vectors pKT262, pKT263, and PKT264.

APPENDIX F

CONTAINMENT CONDITIONS FOR CLONING OF GENES CODING FOR THE BIOSYNTHESIS OF MOLECULES TOXIC FOR VERTEBRATES

Appendix F-I. General Information. Appendix F specifies the containment to be used for the deliberate cloning of genes coding for the biosynthesis of molecules toxic for vertebrates. Cloning of genes coding for molecules toxic for vertebrates that have an LD_{50} of less than 100 nanograms per kilogram body weight (e.g., microbial toxins such as the botulinum toxins, tetanus toxin, diphtheria toxin, Shigella dysenteriae neurotoxin) is prohibited. No specific restrictions shall apply to the cloning of genes if the protein specified by the gene has an LD_{50} of 100 micrograms or more per kilogram of body weight. Experiments involving genes coding for toxic molecules with an LD_{50} of 100 micrograms or less per kilogram body weight shall be registered with ORDA prior to initiating the experiments. A list of toxic molecules classified as to LD_{50} is available from ORDA. Testing procedures for determining toxicity of toxic molecules not on the list are available from ORDA. The results of such tests shall be forwarded to ORDA, which will consult with an ad hoc working group on toxic molecules prior to inclusion of the molecule on the list. (See Section IV-C-1-b-(2)-(e).)

Appendix F-II. Containment Conditions for Cloning of Toxic Molecule Genes in E. coli K-12.

Appendix F-II-A. Cloning of genes coding for molecules toxic for vertebrates that have an LD_{50} in the range of 100 nanograms to 1000 nanograms per kilogram body weight (e.g., abrin, Clostridium perfringens epsilon toxin) may proceed under P2 + EK2 or P3 + EK1 containment conditions.

Appendix F-II-B. Cloning of genes for the biosynthesis of molecules toxic for vertebrates with an LD_{50} in the range of 1 microgram to 100 micrograms per kilogram body weight may proceed under P1 + EK1 containment conditions (e.g., Staphylococcus aureus alpha toxin, Staphylococcus aureus beta toxin, ricin, Pseudomonas aeruginosa exotoxin A, Bordatella pertussis toxin, the lethal factor of Bacillus anthracis, the Pasteurella pestis murine toxins, the oxygen-labile hemolysins such as streptolysin O, and certain neurotoxins present in snake venoms and other venoms).

Appendix F-II-C. Some enterotoxins are substantially more toxic when administered enterally than parenterally. The following enterotoxins shall be subject to P1 + EK1 containment conditions: cholera toxin, the heat labile toxins of E. coli, Klebsiella, and other related proteins that may be identified by neutralization with an antiserum monospecific for cholera toxin, and the heat stable toxins of E. coli and of Yersinia enterocolitica.

Appendix F-III. Containment Conditions for Cloning of Toxic Molecule Genes in Organisms Other than E. coli K-12. Requests involving the cloning of genes coding for molecules toxic for vertebrates in host-vector systems other than E. coli K-12 will be evaluated by ORDA, which will consult with the ad hoc working group on toxic molecules. (See Section IV-C-1-b-(3)-(f).)

Appendix F-IV. Specific Approvals.

Appendix F-IV-A. Permission is granted to clone the Exotoxin A gene of Pseudomonas aeruginosa under P1 conditions in Pseudomonas aeruginosa.

Appendix F-IV-B. The pyrogenic endotoxin type A (Tox A) gene of Staphylococcus aureus may be cloned in an HV2 Bacillus subtilis host-vector system under P3 containment conditions.

Appendix F-IV-C. Permission is granted to clone in E. coli K-12, in high containment Building 550 at the Frederick Cancer Research Facility, restriction fragments of Corynephage Beta carrying the structural gene for diphtheria toxin. Laboratory practices and containment equipment are to be specified by the IBC.

Appendix F-IV-D. The genes coding for the Staphylococcus aureus determinants, A, B, and F, which may be implicated in toxic shock syndrome, may be cloned in E. coli K-12 under P2 + EK1 conditions. The Staphylococcus aureus strain used as the donor is to be alpha toxin minus. It is suggested that, if possible, the donor Staphylococcus aureus strain should lack other toxins with LD_{50}s in the range of one microgram per kilogram body weight, such as the exfoliative toxin.

Appendix F-IV-E. Fragments F-1, F-2, and F-3 of the diphtheria toxin gene (tox) may be cloned in E. coli K-12 under P1 + EK1 containment conditions. Fragment F-1 and fragment F-2 both contain (i) some or all of the transcriptional control elements of tox, (ii) the signal peptide, and (iii) fragment A (the center responsible for ADP-ribosylation of elongation factor 2). Fragment F-3 codes for most of the non-toxic fragment B of the toxin, and contains no sequences coding for any portion of the enzymatically-active fragment A moiety.

Appendix F-IV-F. The gene(s) coding for a toxin (designated LT-like) isolated from E. coli which is similar to the E. coli heat labile enterotoxin (LT) with respect to its activities and mode of action, but is not neutralized by antibodies against cholera enterotoxin or against LT from human or porcine E. coli strains and sequences homologous to the E. coli LT-like toxin gene may be cloned under P1 + EK1 conditions.

Appendix F-IV-G. Genes from Vibrio fluvialis, Vibrio mimicus and non O-1 Vibrio cholerae, specifying virulence factors for animals, may be cloned under P1 + EK1 conditions. The virulence factors to be cloned will be selected by testing fluid induction in suckling mice and Y-1 mouse adrenal cells.

APPENDIX G

PHYSICAL CONTAINMENT

Appendix G-I. Standard Practices and Training. The first principle of containment is a strict adherence to good microbiological practices [1-10]. Consequently, all personnel directly or indirectly involved in experiments on recombinant DNAs must receive adequate instruction. (See Sections IV-B-1-e and IV-B-5-d.) This shall, as a minimum, include instructions in aseptic techniques and in the biology of the organisms used in the experiments, so that the potential biohazards can be understood and appreciated.

Any research group working with agents with a known or potential biohazard shall have an emergency plan which describes the procedures to be followed if an accident contaminates personnel or the environment. The principal investigator must ensure that everyone in the laboratory is familiar with both the potential hazards of the work and the emergency plan. (See Sections IV-B-3-d and IV-B-5-e.) If a research group is working with a known pathogen where there is an effective vaccine it should be made available to all workers. Where serological monitoring is clearly appropriate it shall be provided. (See Section IV-B-1-f.)

The "Laboratory Safety Monograph", available from ORDA, describes practices, equipment, and facilities in detail.

Apendix G-II. Physical Containment Levels. The objective of physical containment is to confine organisms containing recombinant DNA molecules, and thus to reduce the potential for exposure of the laboratory worker, persons outside of the laboratory, and the environment to organisms containing recombinant DNA molecules. Physical containment is achieved through the use of laboratory practices, containment equipment, and special laboratory design. Emphasis is placed on primary means of physical containment which are provided by laboratory practices and containment equipment. Special laboratory design provides a secondary means of protection against the accidental release of organisms outside the laboratory or to the environment. Special laboratory design is used primarily in facilities in which experiments of moderate to high potential hazards are performed.

Combinations of laboratory practices, containment equipment, and special laboratory design can be made to achieve different levels of physical containment. Four levels of physical containment, which are designated as P1, P2, P3, and P4, are described. It should be emphasized that the descriptions and assignments of physical containment detailed below are based on existing approaches to containment of pathogenic organisms. For example, the "Classification of Etiologic Agents on the Basis of Hazard," [2] prepared by the Centers for Disease Control, describes four general levels which roughly correspond to our descriptions for P1, P2, P3, and P4; and the National Cancer Institute describes three levels for research on oncogenic viruses which roughly correspond to our P2, P3, and P4 levels. [3]

It is recognized that several different combinations of laboratory practices, containment equipment, and special laboratory design may be appropriate for containment of specific research activities. The Guidelines, therefore, allow alternative selections of primary containment equipment within facilities that have been designed to provide P3 and P4 levels of physical containment. The selection of alternative methods of primary containment is dependent, however, on the level of biological containment provided by the host-vector system used in the experiment. Consideration will also be given by the Director, NIH, with the advice of the Recombinant DNA Advisory Committee to other combinations which achieve an equivalent level of containment. (See Section IV-C-1-b-(2)-(b).)

Appendix G-II-A. P1 Level.

Appendix G-II-A-1. Laboratory Practices.

Appendix G-II-A-1-a. Laboratory doors shall be kept closed while experiments are in progress.

Appendix G-II-A-1-b. Work surfaces shall be decontaminated daily, and immediately following spills of organisms containing recombinant DNA molecules.

Appendix G-II-A-1-c. All biological wastes shall be decontaminated before disposal. Other contaminated materials, such as glassware, animal cages, and laboratory equipment, shall be decontaminated before washing, reuse, or disposal.

Appendix G-II-A-1-d. Mechanical pipetting devices shall be used; pipetting by mouth is prohibited.

Appendix G-II-A-1-e. Eating, drinking, smoking, and storage of foods are not permitted in the laboratory area in which recombinant DNA materials are handled.

Appendix G-II-A-1-f. Persons shall wash their hands after handling organisms containing recombinant DNA molecules and when they leave the laboratory.

Appendix G-II-A-1-g. Care shall be taken in the conduct of all procedures to minimize the creation of aerosols.

Appendix G-II-A-1-h. Contaminated materials that are to be decontaminated at a site away from the laboratory shall be placed in a durable leakproof container, which is closed before removal from the laboratory.

Appendix G-II-A-1-i. An insect and rodent control program shall be instituted.

Appendix G-II-A-1-j. The use of laboratory gowns, coats, or uniforms is discretionary with the laboratory supervisor.

Appendix G-II-A-1-k. Use of the hypodermic needle and syringe shall be avoided when alternative methods are available.

Appendix G-II-A-1-l. The laboratory shall be kept neat and clean.

Appendix G-II-A-2. Containment Equipment. Special containment equipment is not required at the P1 level.

Appendix G-II-A-3. Special Laboratory Design. Special laboratory design is not required at the P1 level.

Appendix G-II-B. P2 Level.

Appendix G-II-B-1. Laboratory Practices.

Appendix G-II-B-1-a. Laboratory doors shall be kept closed while experiments are in progress.

Appendix G-II-B-1-b. Work surfaces shall be decontaminated daily, and immediately following spills of organisms containing recombinant DNA molecules.

Appendix G-II-B-1-c. All laboratory wastes shall be steam-sterilized (autoclaved) before disposal. Other contaminated materials such as glassware, animal cages, laboratory equipment, and radioactive wastes shall be decontaminated by a means demonstrated to be effective before washing, reuse, or disposal.

Appendix G-II-B-1-d. Mechanical pipetting devices shall be used; pipetting by mouth is prohibited.

Appendix G-II-B-1-e. Eating, drinking, smoking, and storage of food are not permitted in the laboratory area in which recombinant DNA materials are handled.

Appendix G-II-B-1-f. Persons shall wash their hands after handling organisms containing recombinant DNA molecules and when they leave the laboratory.

Appendix G-II-B-1-g. Care shall be exercised to minimize the creation of aerosols. For example, manipulations such as inserting a hot inoculating loop or needle into a culture, flaming an inoculation loop or needle so that it splatters, and forceful ejection of fluids from pipettes or syringes shall be avoided.

Appendix G-II-B-1-h. Contaminated materials that are to be steam sterilized (autoclaved) or decontaminated at a site away from the laboratory shall be placed in a durable leak-proof container, which is closed before removal from the laboratory.

Appendix G-II-B-1-i. Only persons who have been advised of the nature of the research being conducted shall enter the laboratory.

Appendix G-II-B-1-j. The universal biohazard sign shall be posted on all laboratory access doors when experiments requiring P2 containment are in progress. Freezers and refrigerators or other units used to store organisms containing recombinant DNA molecules shall also be posted with the universal biohazard sign.

Appendix G-II-B-1-k. An insect and rodent control program shall be instituted.

Appendix G-II-B-1-l. The use of laboratory gowns, coats, or uniforms is required. Laboratory clothing shall not be worn to the lunch room or outside of the building in which the laboratory is located.

Appendix G-II-B-1-m. Animals not related to the experiment shall not be permitted in the laboratory.

Appendix G-II-B-1-n. Use of the hypodermic needle and syringe shall be avoided when alternative methods are available.

Appendix G-II-B-1-o. The laboratory shall be kept neat and clean.

Appendix G-II-B-1-p. Experiments of lesser biohazard potential can be carried out concurrently in carefully demarcated areas of the same laboratory.

Appendix G-II-B-2. Containment Equipment. Biological safety cabinets [12] shall be used to contain aerosol-producing equipment, such as blenders, lyophilizers, sonicators, and centrifuges, when used to process organisms containing recombinant DNA molecules, except where equipment design provides for containment of the potential aerosol. For example, a centrifuge may be operated in the open if a sealed head or safety centrifuge cups are used.

Appendix G-II-B-3. Special Laboratory Design. An autoclave for sterilization of wastes and contaminated materials shall be available in the same building in which organisms containing recombinant DNA molecules are used.

Appendix G-II-C. P3 Level.

Appendix G-II-C-1. Laboratory Practices.

Appendix G-II-C-1-a. Laboratory doors shall be kept closed while experiments are in progress.

Appendix G-II-C-1-b. Work surfaces shall be decontaminated following the completion of the experimental activity, and immediately following spills of organisms containing recombinant DNA molecules.

Appendix G-II-C-1-c. All laboratory wastes shall be steam-sterilized (autoclaved) before disposal. Other contaminated materials, such as glassware, animal cages, laboratory equipment, and radioactive wastes, shall be decontaminated by a method demonstrated to be effective before washing, reuse, or disposal.

Appendix G-II-C-1-d. Mechanical pipetting devices shall be used; pipetting by mouth is prohibited.

Appendix G-II-C-1-e. Eating, drinking, smoking, and storage of food are not permitted in the laboratory area in which recombinant DNA materials are handled.

Appendix G-II-C-1-f. Persons shall wash their hands after handling organisms containing recombinant DNA molecules and when they leave the laboratory.

Appendix G-II-C-1-g. Care shall be exercised to minimize the creation of aerosols. For example, manipulations such as inserting a hot inoculating loop or needle into a culture, flaming an inoculation loop or needle so that it splatters, and forceful ejection of fluids from pipettes or syringes shall be avoided.

Appendix G-II-C-1-h. Contaminated materials that are to be steam-sterilized (autoclaved) or decontaminated at a site away from the laboratory shall be placed in a durable leak-proof container, which is closed before removal from the laboratory.

Appendix G-II-C-1-i. Entry into the laboratory shall be through a controlled access area. Only persons who have been advised of the nature of the research being conducted shall enter the controlled access area. Only persons required on the basis of program or support needs shall be authorized to enter the laboratory. Such persons shall be advised of the nature of the research being conducted before entry, and shall comply with all required entry and exit procedures.

Appendix G-II-C-1-j. Persons under 16 years of age shall not enter the laboratory.

Appendix G-II-C-1-k. The universal biohazard sign shall be posted on the controlled access area door and on all laboratory doors when experiments requiring P3-level containment are in progress. Freezers and refrigerators

or other units used to store organisms containing recombinant DNA molecules shall also be posted with the universal biohazard sign.

Appendix G-II-C-1-l. An insect and rodent control program shall be instituted.

Appendix G-II-C-1-m. Laboratory clothing that protects street clothing (e.g., long-sleeve solid-front or wrap-around gowns, no-button or slipover jackets) shall be worn in the laboratory. Front-button laboratory coats are unsuitable. Laboratory clothing shall not be worn outside the laboratory and shall be decontaminated before it is sent to the laundry.

Appendix G-II-C-1-n. Raincoats, overcoats, topcoats, coats, hats, caps, and such street outer-wear shall not be kept in the laboratory.

Appendix G-II-C-1-o. Gloves shall be worn when handling materials requiring P3 containment. They shall be removed aseptically immediately after the handling procedure and decontaminated.

Appendix G-II-C-1-p. Animals and plants not related to the experiment shall not be permitted in the laboratory.

Appendix G-II-C-1-q. Vacuum outlets shall be protected by filter and liquid disinfectant traps.

Appendix G-II-C-1-r. Use of hypodermic needle and syringe shall be avoided when alternative methods are available.

Appendix G-II-C-1-s. The laboratory shall be kept neat and clean.

Appendix G-II-C-1 t. If experiments involving other organisms which require lower levels of containment are to be conducted in the same laboratory concurrently with experiments requiring P3-level physical containment, they shall be conducted in accordance with all P3-level laboratory practices.

Appendix G-II-C-2. Containment Equipment.

Appendix G-II-C-2-a. Biological safety cabinets [12] shall be used for all equipment and manipulations that produce aerosols -- e.g., pipetting, dilutions, transfer operations, plating, flaming, grinding, blending, drying, sonicating, shaking, centrifuging -- where these procedures involve organisms containing recombinant DNA molecules, except where equipment design provides for containment of the potential aerosol.

Appendix G-II-C-2-b. Laboratory animals held in a P3 area shall be housed in partial-containment caging systems, such as Horsfall units [11], open cages placed in ventilated enclosures, solid-wall and -bottom cages covered by filter bonnets, or solid-wall and -bottom cages placed on holding racks equipped with ultraviolet radiation lamps and reflectors. (Note: Conventional caging systems may be used, provided that all personnel wear appropriate personal protective devices. These shall include, at a minimum, wrap-around gowns, head covers, gloves, shoe covers, and respirators. All personnel shall shower on exit from areas where these devices are required.)

Appendix G-II-C-2-c. Alternative Selection of Containment Equipment. Experimental procedures involving a host-vector system that provides a one-step higher level of biological containment than that specified can be conducted in the P3 laboratory using containment equipment specified for the P2 level of physical containment. Experimental procedures involving a host-vector system that provides a one-step lower level of biological containment than that specified can be conducted in the P3 laboratory using containment equipment specified for the P4 level of physical containment. Alternative combinations of containment safeguards are shown in Table I.

Table I

POSSIBLE COMBINATIONS OF CONTAINMENT SAFEGUARDS

Classification of experiment		Alternate combinations of physical and biological containment			
		Physical Containment			
Physical containment	Biological* containment	Laboratory design specified for:	Laboratory practices specified for:	Containment equipment specified for:	Biological containment
P3	HV2	P3	P3	P3	HV2
P3	HV2	P3	P3	P4	HV1
P3	HV1	P3	P3	P3	HV1
P3	HV1	P3	P3	P2	HV2

*See Appendix I for description of biological containment.

Appendix G-II-C-3. Special Laboratory Design.

Appendix G-II-C-3-a. The laboratory shall be separated by a controlled access area from areas that are open to unrestricted traffic flow. A controlled access area is an anteroom, a change room, an air lock or any other double-door arrangement that separates the laboratory from areas open to unrestricted traffic flow.

Appendix G-II-C-3-b. The surfaces of walls, floors, and ceilings shall be readily cleanable. Penetrations through these surfaces shall be sealed or capable of being sealed to facilitate space decontamination.

Appendix G-II-C-3-c. A foot-, elbow-, or automatically-operated hand-washing facility shall be provided near each primary laboratory exit area.

Appendix G-II-C-3-d. Windows in the laboratory shall be sealed.

Appendix G-II-C-3-e. An autoclave for sterilization of wastes and contaminated materials shall be available in the same building (and preferably within the controlled laboratory area) in which organisms containing recombinant DNA molecules are used.

Appendix G-II-C-3-f. The laboratory shall have a ventilation system that is capable of controlling air movement. The movement of air shall be from areas of lower contamination potential to areas of higher contamination

potential (i.e., from the controlled access area to the laboratory area). If the ventilation system provides positive pressure supply air, the system shall operate in a manner that prevents the reversal of the direction of air movement or shall be equipped with an alarm that would be actuated in the event that reversal in the direction of air movement were to occur. The exhaust air from the laboratory area shall not be recirculated to other areas of the building unless the exhaust air is filtered by HEPA filters or equivalent. The exhaust air from the laboratory area can be discharged to the outdoors without filtration or other means for effectively reducing an accidental aerosol burden provided that it can be dispersed clear of occupied buildings and air intakes.

Appendix G-II-C-3-g. The treated exhaust-air from Class I and Class II biological safety cabinets [12] may be discharged either to the laboratory or to the outdoors. The treated exhaust-air from a Class III cabinet shall be discharged directly to the outdoors. If the treated exhaust-air from these cabinets is to be discharged to the outdoors through a building exhaust air system, it shall be connected to this system so as to avoid any interference with the air balance of the cabinet and the building ventilation system.

Appendix G-II-D. P4 Level.

Appendix G-II-D-1. Laboratory Practices.

Appendix G-II-D-1-a. Laboratory doors shall be kept closed while experiments are in progress.

Appendix G-II-D-1-b. Work surfaces shall be decontaminated following the completion of the experimental activity and immediately following spills of organisms containing recombinant DNA molecules.

Appendix G-II-D-1-c. All laboratory wastes shall be steam-sterilized (autoclaved) before disposal. Other contaminated materials such as glassware, animal cages, laboratory equipment, and radioactive wastes shall be decontaminated by a method demonstrated to be effective before washing, reuse, or disposal.

Appendix G-II-D-1-d. Mechanical pipetting devices shall be used; pipetting by mouth is prohibited.

Appendix G-II-D-1-e. Eating, drinking, smoking, and storage of food are not permitted in the P4 facility.

Appendix G-II-D-1-f. Persons shall wash their hands after handling organisms containing recombinant DNA molecules and when they leave the laboratory.

Appendix G-II-D-1-g. Care shall be exercised to minimize the creation of aerosols. For example, manipulations such as inserting a hot inoculating loop or needle into a culture, flaming an inoculation loop or needle so that

it splatters, and forceful ejection of fluids from pipettes or syringes shall be avoided.

Appendix G-II-D-1-h. Biological materials to be removed from the P4 facility in a viable or intact state shall be transferred to a nonbreakable sealed container, which is then removed from the P4 facility through a pass-through disinfectant dunk tank or fumigation chamber.

Appendix G-II-D-1-i. No materials, except for biological materials that are to remain in a viable or intact state, shall be removed from the P4 facility unless they have been steam-sterilized (autoclaved) or decontaminated by a means demonstrated to be effective as they pass out of the P4 facility. All wastes and other materials as well as equipment not damaged by high temperature or steam shall be steam sterilized in the double-door autoclave of the P4 facility. Other materials which may be damaged by temperature or steam shall be removed from the P4 facility through a pass-through fumigation chamber.

Appendix G-II-D-1-j. Materials within the Class III cabinets shall be removed from the cabinet system only after being steam-sterilized in an attached double-door autoclave or after being contained in a nonbreakable sealed container, which is then passed through a disinfectant dunk tank or a fumigation chamber.

Appendix G-II-D-1-k. Only persons whose entry into the P4 facility is required to meet program or support needs shall be authorized to enter. Before entering, such persons shall be advised of the nature of the research being conducted and shall be instructed as to the appropriate safeguards to ensure their safety. They shall comply with instructions and all other required procedures.

Appendix G-II-D-1-l. Persons under 18 years of age shall not enter the P4 facility.

Appendix G-II-D-1-m. Personnel shall enter into and exit from the P4 facility only through the clothing change and shower rooms. Personnel shall shower at each egress from the P4 facility. Air locks shall not be used for personnel entry or exit except for emergencies.

Appendix G-II-D-1-n. Street clothing shall be removed in the outer side of the clothing-change area and kept there. Complete laboratory clothing, including undergarments, head cover, shoes, and either pants and shirts or jumpsuits, shall be used by all persons who enter the P4 facility. Upon exit, personnel shall store this clothing in lockers provided for this purpose or discard it into collection hampers before entering the shower area.

Appendix G-II-D-1-o. The universal biohazard sign is required on the P4 facility access doors and on all interior doors to individual laboratory rooms where experiments are conducted. The sign shall also be posted on

freezers, refrigerators, or other units used to store organisms containing recombinant DNA molecules.

Appendix G-II-D-1-p. An insect and rodent control program shall be instituted.

Appendix G-II-D-1-q. Animals and plants not related to the experiment shall not be permitted in the laboratory in which the experiment is being conducted.

Appendix G-II-D-1-r. Vacuum outlets shall be protected by filter and liquid disinfectant traps.

Appendix G-II-D-1-s. Use of the hypodermic needle and syringe shall be avoided when alternate methods are available.

Appendix G-II-D-1-t. The laboratory shall be kept neat and clean.

Appendix G-II-D-1-u. If experiments involving other organisms which require lower levels of containment are to be conducted in the P4 facility concurrently with experiments requiring P4-level containment, they shall be conducted in accordance with all P4-level laboratory practices specified in this section.

Appendix G-II-D-2. Containment Equipment.

Appendix G-II-D-2-a. Experimental procedures involving organisms that require P4-level physical containment shall be conducted either in (i) a Class III cabinet system or in (ii) Class I or Class II cabinets that are located in a specially designed area in which all personnel are required to wear one-piece positive-pressure isolation suits.

Appendix G-II-B-4-b. Laboratory animals involved in experiments requiring P4-level physical containment shall be housed either in cages contained in Class III cabinets or in partial containment caging systems (such as Horsfall units [11], open cages placed in ventilated enclosures, or solid-wall and -bottom cages covered by filter bonnets, or solid-wall and -bottom cages placed on holding racks equipped with ultraviolet irradiation lamps and reflectors) that are located in a specially designed area in which all personnel are required to wear one-piece positive-pressure suits.

Appendix G-II-D-2-c. Alternative Selection of Containment Equipment. Experimental procedures involving a host-vector system that provides a one-step higher level of biological containment than that specified can be conducted in the P4 facility using containment equipment requirements specified for the P3 level of physical containment. Alternative combinations of containment safeguards are shown in Table II.

Table II

POSSIBLE COMBINATIONS OF CONTAINMENT SAFEGUARDS

Classification of experiment		Alternate combinations of physical and biological containment			
		Physical containment			
Physical containment	Biological* containment	Laboratory design specified for:	Laboratory practices specified for:	Containment equipment specified for:	Biological containment
P4	HV1	P4	P4	P4	HV1
P4	HV1	P4	P4**	P3	HV2

* See Appendix I for description of biological containment.
** In this case gloves shall be worn, in addition to the clothing requirements specified in Appendix G-II-D-1-n.

Appendix G-II-D-3. Special Laboratory Design.

Appendix G-II-D-3-a. The laboratory shall be located in a restricted-access facility which is either a separate building or a clearly demarcated and isolated zone within a building. Clothing-change areas and shower rooms shall be provided for personnel entry and egress. These rooms shall be arranged so that personnel leave through the shower area to the change room. A double-door ventilated vestibule or ultraviolet air lock shall be provided for passage of materials, supplies, and equipment which are not brought into the P4 facility through the change room area.

Appendix G-II-D-3-b. Walls, floors, and ceilings of the P4 facility are constructed to form an internal shell which readily allows vapor-phase decontamination and is animal- and insect-proof. All penetrations through these structures and surfaces are sealed. (The integrity of the walls, floors, ceilings, and penetration seals should ensure adequate containment of a vapor-phase decontaminant under static pressure conditions. This requirement does not imply that these surfaces must be airtight.)

Appendix G-II-D-3-c. A foot-, elbow-, or automatically-operated hand-washing facility shall be provided near the door within each laboratory in which experiments involving recombinant DNA are conducted in openface biological safety cabinets.

Appendix G-II-D-3-d. Central vacuum systems are permitted. The system, if provided, shall not serve areas outside the P4 facility. The vacuum system shall include in-line HEPA filters near each use point or service cock. The filters shall be installed so as to permit in-place decontamination and replacement. Water supply, liquid and gaseous services provided to the P4 facility shall be protected by devices that prevent backflow.

Appendix G-II-D-3-e. Drinking water fountains shall not be installed in laboratory or animal rooms of the P4 facility. Foot-operated water fountains are permitted in the corridors of the P4 facility. The water

service provided to such fountains shall be protected from the water services to the laboratory areas of the P4 facility.

Appendix G-II-D-3-f. Laboratory doors shall be self-closing.

Appendix G-II-D-3-g. A double-door autoclave shall be provided for sterilization of material passing out of the P4 facility. The autoclave doors shall be interlocked so that both doors will not be open at the same time.

Appendix G-II-D-3-h. A pass-through dunk tank or fumigation chamber shall be provided for removal from the P4 facility of material and equipment that cannot be heat-sterilized.

Appendix G-II-D-3-i. All liquid effluents from the P4 facility shall be collected and decontaminated before disposal. Liquid effluents from biological safety cabinets and laboratory sinks shall be sterilized by heat. Liquid effluents from the shower and hand washing facilities may be activated by chemical treatment. HEPA filters shall be installed in all vents from effluent drains.

Appendix G-II-D-3-j. An individual supply and exhaust-air ventilation system shall be provided. The system shall maintain pressure differentials and directional air flow as required to ensure inflow from areas outside the facility toward areas of highest potential risk within the facility. The system shall be designed to prevent the reversal of air flow. The system shall sound an alarm in the event of system malfunction.

Appendix G-II-D-3-k. Air within individual laboratories of the P4 facility may be recirculated if HEPA filtered.

Appendix G-II-D-3-l. The exhaust air from the P4 facility shall be HEPA filtered and discharged to the outdoors so that it is dispersed clear of occupied buildings and air intakes. The filter chambers shall be designed to allow in situ decontamination before removal and to facilitate certification testing after replacement.

Appendix G-II-D-3-m. The treated exhaust-air from Class I and Class II biological safety cabinets [12] may be discharged directly to the laboratory room environment or to the outdoors. The treated exhaust-air from Class III cabinets shall be discharged to the outdoors. If the treated exhaust-air from these cabinets is to be discharged to the outdoors through the P4 facility exhaust air system, it shall be connected to this system so as to avoid any interference with the air balance of the cabinets or the facility exhaust air system.

Appendix G-II-D-3-n. As noted in Appendix G-II-D-2-a, the P4 facility may contain specially designed areas in which all personnel are required to wear one-piece positive-pressure isolation suits. Such areas shall be air-tight. The exhaust-air from the suit area shall be filtered by two sets of HEPA filters installed in series, and a duplicate filtration unit and exhaust fan shall be provided. The air pressure within the suit area shall be less than that on any adjacent area. An emergency lighting system, communication

systems, and power source shall be provided. A double-door autoclave shall be provided for sterilization of all waste materials to be removed from the suit area.

Personnel who enter this area shall wear a one-piece positive-pressure suit that is ventilated by a life-support system. The life-support system shall be provided with alarms and emergency backup air. Entry to this area is through an airlock fitted with airtight doors. A chemical shower area shall be provided to decontaminate the surfaces of the suit before removal.

Appendix G-III. Footnotes and References of Appendix G.

1. Laboratory Safety at the Center for Disease Control (Sept. 1974). U.S. Department of Health Education and Welfare Publication No. CDC 75-8118.

2. Classification of Etiologic Agents on the Basis of Hazard. (4th Edition, July 1974). U.S. Department of Health, Education and Welfare. Public Health Service. Centers for Disease Control, Office of Biosafety, Atlanta, Georgia 30333.

3. National Cancer Institute Safety Standards for Research Involving Oncogenic Viruses (Oct. 1974). U.S. Department of Health, Education and Welfare Publication No. (NIH) 75-790.

4. National Institutes of Health Biohazards Safety Guide (1974). U.S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health. U.S. Government Printing Office, Stock No. 1740-00383.

5. Biohazards in Biological Research (1973). A. Hellman, M. N. Oxman, and R. Pollack (ed.) Cold Spring Harbor Laboratory.

6. Handbook of Laboratory Safety (1971). Second Edition. N. V. Steere (ed.). The Chemical Rubber Co., Cleveland.

7. Bodily, J. L. (1970). General Administration of the Laboratory, H. L. Bodily, E. L. Updyke, and J. O. Mason (eds.), Diagnostic Procedures for Bacterial, Mycotic and Parasitic Infections. American Public Health Association, New York, pp. 11-28.

8. Darlow, H. M. (1969). Safety in the Microbiological Laboratory. In J. R. Norris and D. W. Robbins (ed.), Methods in Microbiology. Academic Press, Inc. New York. pp. 169-204.

9. The Prevention of Laboratory Acquired Infection (1974). C. H. Collins, E. G. Hartley, and R. Pilsworth. Public Health Laboratory Service, Monograph Series No. 6.

10. Chatigny, M. A. (1961). Protection Against Infection in the Microbiological Laboratory: Devices and Procedures. In W. W. Umbreit (ed.). Advances in Applied Microbiology. Academic Press, New York, N.Y. 3:131-192.

11. Horsfall, F. L., Jr., and J. H. Baner (1940). Individual Isolation of Infected Animals in a Single Room. J. Bact. 40, 569-580.

12. Biological safety cabinets referred to in this section are classified as Class I, Class II, or Class III cabinets. A Class I is a ventilated cabinet for personnel protection having an inward flow of air away from the operator. The exhaust air from this cabinet is filtered through a high-efficiency particulate air (HEPA) filter. This cabinet is used in three operational modes: (1) with a full-width open front, (2) with an installed front closure panel (having four 8-inch diameter openings) without gloves, and (3) with an installed front closure panel equipped with arm-length rubber gloves. The face velocity of the inward flow of air through the full-width open front is 75 feet per minute or greater.

A Class II cabinet is a ventilated cabinet for personnel and product protection having an open front with inward air flow for personnel protection, and HEPA filtered mass recirculated air flow for product protection. The cabinet exhaust air is filtered through a HEPA filter. The face velocity of the inward flow of air through the full-width open front is 75 feet per minute or greater. Design and performance specifications for Class II cabinets have been adopted by the National Sanitation Foundation, Ann Arbor, Michigan. A Class III cabinet is a closed-front ventilated cabinet of gas-tight construction which provides the highest level of personnel protection of all biohazard safety cabinets. The interior of the cabinet is protected from contaminants exterior to the cabinet. The cabinet is fitted with arm-length rubber gloves and is operated under a negative pressure of at least 0.5 inches water gauge. All supply air is filtered through HEPA filters. Exhaust air is filtered through two HEPA filters or one HEPA filter and incinerator before being discharged to the outside environment.

APPENDIX H

SHIPMENT

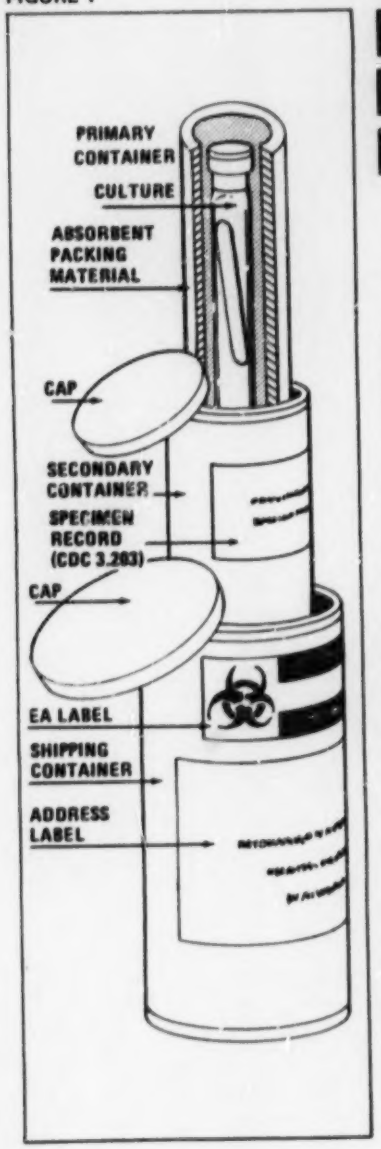
Recombinant DNA molecules contained in an organism or virus shall be shipped only as an etiologic agent under requirements of the U.S. Public Health Service, and the U.S. Department of Transportation (Section 72.3, Part 72, Title 42, and Sections 173.386-.388, Part 173, Title 49, U.S. Code of Federal Regulations (CFR)) as specified below:

Appendix H-I. Recombinant DNA molecules contained in an organism or virus requiring P1, P2, or P3 physical containment, when offered for transportation or transported, are subject to all requirements of Section 72.3(a)-(e), Part 72, Title 42 CFR, and Sections 173.386-.388, Part 173, Title 49 CFR.

Appendix H-II. Recombinant DNA molecules contained in an organism or virus requiring P4 physical containment, when offered for transportation or transported, are subject to the requirements listed above under Appendix H-I and are also subject to Section 72.3(f), Part 72, Title 42 CFR.

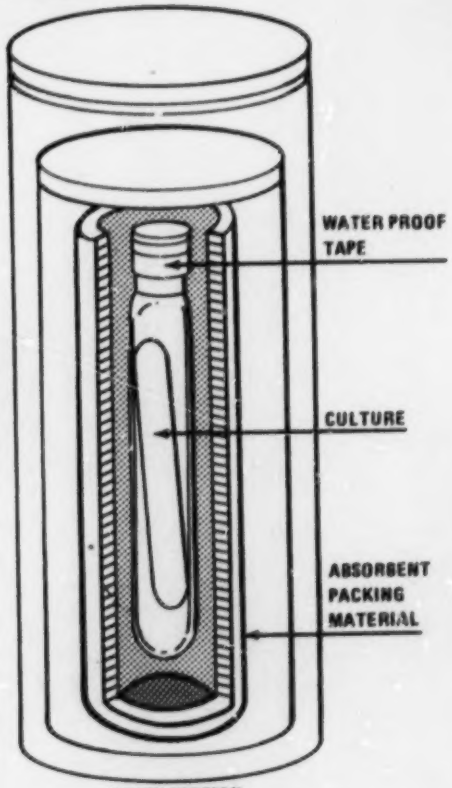
Appendix H-III. Information on packaging and labeling of etiologic agents is shown in Figures 1, 2, and 3. Additional information on packaging and shipment is given in the "Laboratory Safety Monograph - A Supplement to the NIH Guidelines for Recombinant DNA Research," available from ORDA.

FIGURE 1



PACKAGING AND LABELING OF ETIOLOGIC AGENTS

FIGURE 2



CROSS SECTION OF PROPER PACKING

The Interstate Shipment of Etiologic Agents (42 CFR, Part 72) was revised July 21, 1980 to provide for packaging and labeling requirements for etiologic agents and certain other materials shipped in interstate traffic.

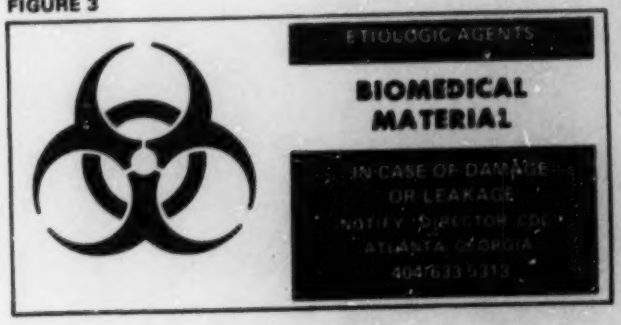
Figures 1 and 2 diagram the packaging and labeling of etiologic agents in volumes of less than 50 ml. in accordance with the provisions of subparagraph 72.3 (a) of the cited regulation. Figure illustrates the color and size of the label, described in subparagraph 72.3 (d) (1 - 5) of the regulations, which shall be affixed to all shipments of etiologic agents.

For further information on any provision of this regulation contact:

Centers for Disease Control
Attn: Biohazards Control Office
1600 Clifton Road
Atlanta, Georgia 30333

Telephone: 404—329-3883
FTS—236-3883

FIGURE 3



APPENDIX I

BIOLOGICAL CONTAINMENT

Appendix I-I. Levels of Biological Containment. In consideration of biological containment, the vector (plasmid, organelle, or virus) for the recombinant DNA and the host (bacterial, plant, or animal cell) in which the vector is propagated in the laboratory will be considered together. Any combination of vector and host which is to provide biological containment must be chosen or constructed so that the following types of "escape" are minimized: (i) survival of the vector in its host outside the laboratory and (ii) transmission of the vector from the propagation host to other nonlaboratory hosts.

The following levels of biological containment (HV, or Host-Vector, systems) for prokaryotes will be established; specific criteria will depend on the organisms to be used.

Appendix I-I-A. HV1. A host-vector system which provides a moderate level of containment. Specific systems:

Appendix I-I-A-1. EK1. The host is always E. coli K-12 or a derivative thereof, and the vectors include nonconjugative plasmids (e.g., pSC101, ColE1, or derivatives thereof [1-7]) and variants of bacteriophage, such as lambda [8-15]. The E. coli K-12 hosts shall not contain conjugation-proficient plasmids, whether autonomous or integrated, or generalized transducing phages.

Appendix I-I-A-2. Other HV1. Hosts and vectors shall be, at a minimum, comparable in containment to E. coli K-12 with a non conjugative plasmid or bacteriophage vector. The data to be considered and a mechanism for approval of such HV1 systems are described below (Appendix I-II).

Appendix I-I-B. HV2. These are host-vector systems shown to provide a high level of biological containment as demonstrated by data from suitable tests performed in the laboratory. Escape of the recombinant DNA either via survival of the organisms or via transmission of recombinant DNA to other organisms should be less than $1/10^8$ under specified conditions. Specific systems:

Appendix I-I-B-1. For EK2 host-vector systems in which the vector is a plasmid, no more than one in 10^8 host cells should be able to perpetuate a cloned DNA fragment under the specified nonpermissive laboratory conditions designed to represent the natural environment, either by survival of the original host or as a consequences of transmission of the cloned DNA fragment.

Appendix I-I-B-2. For EK2 host-vector systems in which the vector is a phage, no more than one in 10^8 phage particles should be able to perpetuate a cloned DNA fragment under the specified nonpermissive laboratory conditions designed to represent the natural environment either (i) as a prophage (in the inserted or plasmid form) in the laboratory host used for phage propagation or (ii) by surviving in natural environments and transferring a cloned DNA fragment to other hosts (or their resident prophages).

Appendix I-II. Certification of Host-Vector Systems.

Appendix I-II-A. Responsibility. HV1 systems other than E. coli K-12, and HV2 host-vector systems, may not be designated as such until they have been certified by the Director, NIH. Application for certification of a host-vector system is made by written application to the Office of Recombinant DNA Activities, National Institutes of Health, Bethesda, Maryland 20205.

Host-vector systems that are proposed for certification will be reviewed by the National Institutes of Health (NIH) Recombinant DNA Advisory Committee (RAC). (See Section IV-C-1-b-(1)-(e).) This will first involve review of the data on construction, properties, and testing of the proposed host-vector system by a Working Group composed of one or more members of the RAC and other persons chosen because of their expertise in evaluating such data. The Committee will then evaluate the report of the Working Group and any other available information at a regular meeting. The Director, NIH, is responsible for certification after receiving the advice of the RAC. Minor modifications of existing certified host-vector systems, where the modifications are of minimal or no consequence to the properties relevant to containment may be certified by the Director, NIH, without review by the RAC. (See Section IV-C-1-b-(3)-(c).)

When new host-vector systems are certified, notice of the certification will be sent by the Office of Recombinant DNA Activities (ORDA) to the applicant

and to all Institutional Biosafety Committees (IBCs) and will be published in the Recombinant DNA Technical Bulletin. Copies of a list of all currently certified host-vector systems may be obtained from ORDA at any time.

The Director, NIH, may at any time rescind the certification of any host-vector system. (See Section IV-C-1-b-(3)-(d).) If certification of a host-vector system is rescinded, NIH will instruct investigators to transfer cloned DNA into a different system, or use the clones at a higher physical containment level unless NIH determines that the already constructed clones incorporate adequate biological containment.

Certification of a given system does not extend to modifications of either the host or vector component of that system. Such modified systems must be independently certified by the Director, NIH. If modifications are minor, it may only be necessary for the investigator to submit data showing that the modifications have either improved or not impaired the major phenotypic traits on which the containment of the system depends. Substantial modifications of a certified system require the submission of complete testing data.

Appendix I-II-B. Data To Be Submitted for Certification.

Appendix I-II-B-1. HV1 Systems Other than E. coli K-12. The following types of data shall be submitted, modified as appropriate for the particular system under consideration. (i) A description of the organism and vector; the strain's

natural habitat and growth requirements; its physiological properties, particularly those related to its reproduction and survival and the mechanisms by which it exchanges genetic information; the range of organisms with which this organism normally exchanges genetic information and what sort of information is exchanged; and any relevant information on its pathogenicity or toxicity. (ii) A description of the history of the particular strains and vectors to be used, including data on any mutations which render this organism less able to survive or transmit genetic information. (iii) A general description of the range of experiments contemplated, with emphasis on the need for developing such an HV1 system.

Appendix I-II-B-2. HV2 Systems. Investigators planning to request HV2 certification for host-vector systems can obtain instructions from ORDA concerning data to be submitted [14-15]. In general, the following types of data are required: (i) Description of construction steps, with indication of source, properties, and manner of introduction of genetic traits. (ii) Quantitative data on the stability of genetic traits that contribute to the containment of the system. (iii) Data on the survival of the host-vector system under nonpermissive laboratory conditions designed to represent the relevant natural environment. (iv) Data on transmissibility of the vector and/or a cloned DNA fragment under both permissive and nonpermissive conditions. (v) Data on all other properties of the system which affect containment and utility, including information on yields of phage or plasmid

molecules, ease of DNA isolation, and ease of transfection or transformation. (vi) In some cases, the investigator may be asked to submit data on survival and vector transmissibility from experiments in which the host-vector is fed to laboratory animals and human subjects. Such in vivo data may be required to confirm the validity of predicting in vivo survival on the basis of in vitro experiments.

Data must be submitted in writing to ORDA. Ten to twelve weeks are normally required for review and circulation of the data prior to the meeting at which such data can be considered by the RAC. Investigators are encouraged to publish their data on the construction, properties, and testing of proposed HV2 systems prior to consideration of the system by the RAC and its subcommittee. More specific instructions concerning the type of data to be submitted to NIH for proposed EK2 systems involving either plasmids or bacteriophage in E. coli K-12 are available from ORDA.

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APPENDIX J

FEDERAL INTERAGENCY ADVISORY COMMITTEE ON RECOMBINANT DNA RESEARCH

Appendix J-I. Federal Interagency Advisory Committee. The Federal Interagency Advisory Committee on Recombinant DNA Research advises the Secretary of the Department of Health and Human Services, the Assistant Secretary for Health, and the Director, National Institutes of Health, on the coordination of those aspects of all Federal programs and activities relating to recombinant DNA research. The Committee provides for communication and exchange of information necessary to maintain adequate coordination of such programs and activities. The Committee is responsible for facilitating compliance with a uniform form set of guidelines in the conduct of this research in the public and private sectors and, where warranted, to suggest administrative or legislative proposals.

The Director of the NIH, or his designee, serves as Chairman, and the Committee includes representation from all Departments and Agencies whose programs involve health functions or responsibilities as determined by the Secretary.

Departments and Agencies which have representation on this Committee, as of December 1980, are:

Department of Agriculture

Department of Commerce

Department of Defense

Department of Energy

Environmental Protection Agency

Executive Office of the President

Department of Health and Human Services

- Office of the Assistant Secretary for Health
- Centers for Disease Control
- Food and Drug Administration
- National Institutes of Health

Department of the Interior

Department of Justice

Department of Labor

National Aeronautics and Space Administration

National Science Foundation

Nuclear Regulatory Commission

Department of State

Department of Transportation

Arms Control and Disarmament Agency

Veterans Administration

At the second meeting of the Committee on November 23, 1976, all of the Federal agencies endorsed the NIH Guidelines, and Departments which support or conduct recombinant DNA research agreed to abide by the NIH Guidelines [1].

Appendix J-II. Footnote of Appendix J.

1. Minutes of the first eight meetings of the Federal Interagency Advisory Committee on Recombinant DNA Research are reproduced in Recombinant DNA Research, Volume 2, Documents Relating to "NIH Guidelines for Research Involving Recombinant DNA Molecules," June 1976 - November 1977.

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